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STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

I. Effect of the Various Factors Composing a Tropical Climate on Growth and Fertility of Mice

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As a preparation for work on the acclimatization of man to a tropical environment I conducted in 1919–20 a number of experiments on the physiological behavior of mice in an artificially produced tropical climate. I hoped by these experiments to gain an insight into the mechanisms of adaptation that are released by transfer to a hot environment. During the course of my investigation and more so after finding an opportunity to continue my work under conditions actually prevailing in the tropics, I have become convinced that a considerable part of research in the climatological physiology of hot countries may advantageously be done in temperate climates. By experiments on animals a broad foundation could be built for this science. When results primarily applicable to man are desired it becomes, naturally, necessary to confirm these results by anthropological observations in the Tropics. When these latter, however, are conducted alone, the difficulty in finding homogeneous material and the lack of synchronous controls will render them difficult to interpret. The ideal procedure in work along these lines is a coöperation of anthropological observation and animal experiment.

So far as I am aware, there does not exist at the present time any investigation on a large scale on animals which have been exposed to external heat for several generations with the physiological effects arising therefrom in view. A considerable number of observations

is reported on the effect of exposures to heat of short duration. For solving certain well-defined physiological problems such methods are adequate. Very little of the information so acquired can, however, be applied to the problems connected with the acclimatization of the white race to a tropical climate. Incomparably more may be learned from series extending for several generations. Longer series have been reported by Przibram (1) on rats and by Sumner (2) on mice but both these investigators have not been concerned about the physiological aspects of the problem. Both have discovered very interesting morphological changes arising from exposure to heat which still await their physiological explanation. Recently Steinach and Kammerer (3) have taken a step in the outlined direction, but their investigation is more especially concerned with the sexual functions. Their material on growth is limited to weighings of the members of *one* litter and is, naturally, entirely inadequate for the purpose.

For the physiological interpretation of environmental experiments it is desirable to maintain as closely as possible a fixed difference in temperature between the hot series and the controls. Sumner paid very little attention to this prerequisite. The difference in temperature between his cold and warm room series was very variable and reached sometimes 18°C. No attention was paid to the humidity of the air. The temperature of the cold room was probably below the optimal range for the physical welfare of mice, which would explain the fact that Sumner failed to discover any appreciable difference in growth between his heat mice and his controls. On the other hand, the Vienna investigators have in their experiments adopted the incubator principle and kept their rats at constant temperature and humidity. As considerable variations may occur in an actual tropical climate and as it has, furthermore, been shown that mice thrive better when the temperature is slightly variable (4) I have in my experiments avoided constant temperatures, but at the same time insisted upon a constant interval between the temperatures in the hot and temperate room.

I was fortunate in securing breeding mice for my investigation from a stock that for five years had been promiscuously bred and thoroughly observed by Professor Robertson and later by Doctor Hagedoorn. The former has published extensive data on the normal growth and fertility of these mice (5). His work has served me as a model. My thanks are due for personal advice and for the encouragement Doctor Robertson has given me. I am also indebted to Doctor Hagedoorn for many suggestions in connection with my work.

Robertson (6) has furnished statistical evidence to show that the variability of his mouse stock decreased progressively. Since the variability of weight of my control animals, as I hope to show in this paper, was still smaller than in Robertson's 1916 series, I venture to maintain that the genetical homogeneity of my mice, at least as far as their size is concerned, was as complete as can be wished for in work of this sort. This homogeneity was further enhanced by the precaution I took, in making up various series, to divide equally a number of litters among them. The total number of animals was in the neighborhood of one thousand. Approximately half of this number were employed for growth studies. The rest were used for various other experiments, the results of which will be reported in subsequent papers.

Although my principal object in view was to compare the growth of mice that either had been brought into the hot room at an early age—as a rule when 3 weeks old—or which were born in the same environment, with the growth of mice that were continuously kept at ordinary room temperature, I added to my program a number of other series which I hoped might elucidate the rôle such climatic factors as light and circulation of air play in the adaptation to a tropical climate. In addition to the control series that was reared in subdued light I kept in the "temperate" room two other series, "immigrants" and "descendants" (when these terms are used in my papers they mean mice that were transferred to the new environment when 3 weeks old and mice that were born there, respectively) in which the animals were exposed to the radiation of strong artificial light. In the hot room two corresponding series of "light" mice were kept. The effect of air in motion was studied only in the hot room and in subdued light. The "hot, still air, subdued light, descendant" series was divided in three subseries corresponding to four succeeding generations of mice. The second of these subseries consisted of mice from the second and third generations, whose growth curves were identical and which, because of small absolute numbers, have been considered together.

The "tropical" room had an air capacity of 6½ cu. m. which is approximately the same as the size of the rooms the Vienna biologists employed for their heat experiments. It was fitted with double walls, which were separated by an air space. It further had a double door and a double window. The inner wall and the inner door were covered with heat insulating material. Although no draught could be detected, the slow passage of air through the walls seemed to be sufficient for ventilation. No appreciable rise of carbon dioxide was found, which probably was

due to the comparatively small number of mice that simultaneously occupied the room.

The heating of the room was accomplished by an electric hot plate, the heat of which could be regulated according to the outside temperature. By exercising some care in observing the temperature changes, especially in summer, I succeeded in keeping the dry bulb temperature of the hot room close to 10°C. above the temperature of the room where the control series were kept. This latter was steam heated in winter. The high humidity of the "tropical" room was in the beginning maintained by a dropping device, drops of distilled water of regulated size falling direct on the hot plate. Later it was found that a large basin filled with water and placed on the hot plate served the same purpose.

The table below embodies observations of the dry and wet bulb readings taken continuously in January and February, 1919.

	DRY BULB TEMPERATURE			WET BULB TEMPERATURE		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Temperate room						
a.m.	22.7	13.0	18.3	16.8	9.3	13.5
p.m.	26.8	21.1	23.7	20.6	16.0	18.2
Average	24.8	17.1	21.0	18.7	12.7	15.9
Hot room						
a.m.	31.5	23.8	29.2	29.7	19.8	25.3
p.m.	38.0	29.4	34.4	33.0	26.0	30.4
Average	34.8	26.6	31.8	31.4	22.9	27.9

Another series of observations taken in 1920 rendered the same dry bulb averages and ranges but a slightly lower humidity. Thermograph records were taken for long periods of time and the results that were calculated from them confirmed the figures obtained from dry bulb observations in the accurate series.

The source of light in the experiments consisted of "Mazda" globes. Two 60 watt lamps were employed in the temperate room and a 100 watt one in the hot room. The globes were suspended about 50 cm. above the bottom of the cages. The light was turned on in the morning and off at night. I admit that my imitation of the tropical sun was far from ideal. I believe, however, that the employment of an arc lamp or other light of a spectral composition that would more closely

have resembled sunlight would, for albino mice, have been contraindicated. I am inclined to the view that the amount of light that penetrated into deeper tissues was as large in these experiments on mice as may occur in man when exposed to the tropical sun. It has been found that tungsten light contains an appreciable percentage of shorter waves. The incipient pigmentation that was observed in a number of "light" mice which will be reported in another paper of this series, and further the indisputable effect of the light on the growth curves of the mice, bear evidence that the light radiation *per se* was capable of producing physiological effects. The different methods the mice invented to protect themselves further proves that they felt strongly the impact of the light. It was, e.g., a common sight to see all the mice in a cage side by side outstretched on their backs with the feet across their bellies.

The ventilating system for the "wind" experiments consisted of an electric wall fan, about 30 cm. in diameter, suspended in horizontal position 25 cm. above the bottom of the cages. The lower end of its case was fitted into a hole in the top of a wooden box, one side of which was removed. The air entered through a cylindrical paste board extension of the fan case that almost reached the ceiling of the room. The air was then blown through the open side of the box in a direction that was opposite to the place where the "still air" cages were kept. A circular air current was thus established. By repeated tests I found that other parts of the room were protected from the draught.

As mouse cages I employed grey enameled wash basins, 11 inches in diameter, which were covered with a wire net, three meshes to an inch. The bottom of the cage was covered with sawdust, which was changed at frequent intervals. In the "light" series the food was put into the cage, in others it was placed on top of the wire net. In the majority of cages about half a dozen animals were confined in each cage. Double this number was sometimes kept in the "light" and "wind" cages without any bad effects on the inmates. In most of these series a stimulation of growth was evident, which would hardly have occurred if any impairment of their physical welfare had resulted from overcrowding.

The food of the mice was strictly homogeneous throughout the investigation and the same for all series. It consisted chiefly of a mush that was freshly prepared every morning of yellow corn meal, 6 parts; rice, 2 parts; rolled barley, 2 parts, and powdered meat scraps, 1 part. The mush was given in the forenoon. In the afternoon a handful of fresh rolled barley was strewn into each cage. Greens were supplied once or twice a week. All the mice had access to water, which was supplied

by the drop-tube method. The normal growth of the control series seems to prove that the mice were properly fed. To insure this I further practised the rule of giving them a surplus of food in excess of their consumption.

The general health of the mice was very good; no cases of infectious diseases occurred in the mouse colony. All the deaths were, as far as I could ascertain, caused either by malnutrition in newly-born mice or by accidental causes such as wounds received in fighting or overheating in older mice. A parasitological survey of a number of feces and intestinal contents revealed nothing exceptional.

The young mice were separated from their mothers when they were 3 weeks old. Since it proved impossible to obtain a sufficient number of "descendant" mice in the hot room from conceptions taking place there, I was compelled to introduce a number of females in a pregnant state in order to fill my "descendant" series.

Because of the meager information available on the effect of the climatic environment on the velocity of growth it has been customary to attribute differences in physical development to nutritional and in man also to sociological factors (7). Certain facts seem to indicate, however, that the climate may play an important rôle in this respect. It has been found in cooler climates that the principal part of the growth of children takes place during summer (8). In some as yet unpublished investigations on the growth of children of Scandinavian parentage in California I observed an acceleration of the third growth cycle in comparison with children from well-to-do classes in Scandinavia in which no undernutrition can be suspected to occur. Zoölogists have further presented evidence to show that species of animals that inhabit a wide area decrease in size toward the equator (9).

As known, the third growth cycle coincides with the maturing of the sexual organs. The time of the first menstruation in females forms a convenient measure of this event. It has been anthropologically and experimentally proven by Steinach and Kammerer (3) that an increase in external temperature stimulates the endocrinal mechanism that regulates the sexual development. These authors have further demonstrated that while a stimulation may take place up to a certain point, beyond this point a retardation may set in. Stefansson (10) has recently added valuable observations that confirm these conceptions. He points, namely, to the fact that menstruation commences in Eskimo girls at an age of 10 to 13 years and attributes this to the tropical temperature that is maintained in the Eskimo huts.

When the heat is combined with high humidity it may be anticipated that the stimulation due to temperature may be counterbalanced by factors working in an opposite direction, primarily by the checking effect the tropical climate has on the output of heat from the body. Sufficient evidence exists to show that several mechanisms may be operating in the interest of the organism to adjust the disproportion between heat production and heat output. The chemical heat regulation, undoubtedly, in small animals plays an important rôle in this respect (11). Some observers contend that the basal metabolism even of man may be diminished in the Tropics (12). The same purpose may be attained by an enlargement of the cooling surface. In the next paper the possibility of modifications of the body capable of producing a larger skin area will be discussed at length. In this connection it suffices to point out that a suppression of growth would answer the same purpose, rendering the body area relatively larger.

Robertson (13) has discussed the statistical methods that enable the biologist to decide whether the number of animals employed is sufficient for the purpose in view. If an accuracy of 1 per cent is required the formula reads

$$N = \sqrt{\frac{100 \times 0.6745 \times \sigma}{M}},$$

where N is the number of variates sought, M the mean and σ the standard deviation. Only a few of my weight series reach this high degree of accuracy. I have therefore deemed it necessary, when comparing the growth curves, to express the difference between each pair of them in terms of the probable error of the same difference. These multiples of the probable errors have been plotted as curves.

The mice were weighed at weekly intervals, in the forenoon before feeding. The weights were recorded to the nearest tenth of a gram. My growth studies cover the principal part of the growth period up to 20 weeks of age. A part of the material was weighed, however, only until they were 12 or 13 weeks old. The growth curves for the animals that were weighed above this age form a direct continuation of the curves for the larger material below the same age, which probably is due to the homogeneity of my mouse colony.

The results of my growth studies are collected in tables 1 to 4. Tables 1 and 2 contain the average weights for each week from the third to the twentieth. Tables 3 and 4 contain data on the variability of the body weights.

The data from tables 1 and 2 are plotted in two graphs, no. 1 for male and no. 2 for female mice. These curves obviously do not require any explanation. Figures 3, 4 and 5 give in form of curves all the data required to add statistical weight to the averages. These data have

TABLE 1

Average weight of male mice

	TEMPERATE ROOM			HOT ROOM						
		Light		Stagnant air						
				Subdued light				Light		Wind
				Descendants						
	Controls	Immigrants	Descendants	Immigrants	1 generation	2 + 3 generations	4 generations	Immigrants	Descendants	
Number weighed	32	24	26	43	56	22	14	11	11	16
age, weeks										
3	6.6	6.0	8.1	7.3	7.4	6.7	6.2	6.3	5.6	7.4
4	9.4	9.7	11.9	9.7	10.1	8.7	8.5	8.4	7.5	9.5
5	12.3	13.0	15.7	11.9	12.0	11.0	12.3	9.1	10.0	11.8
6	14.4	16.4	18.2	13.0	15.1	12.3	14.6	10.8	10.9	13.7
7	16.7	18.2	20.6	13.8	17.2	14.2	16.2		12.8	15.0
8	18.9	19.5	21.9	16.0	18.6	15.8			15.0	17.6
9	20.7	20.3	22.7	17.5	19.9	16.5			17.1	19.1
10	20.7	20.9	23.1	19.0	20.8	17.4			17.2	20.2
11	21.2	21.6	23.5	19.4	20.8	18.2			18.4	22.4
12	21.7	22.0	23.9	20.2	21.9	19.2				22.6
Number weighed	13	21	17	14	19					11
age, weeks										
13	22.1	22.5	24.2	21.2	21.4					22.1
14	23.5	22.8	24.2	21.1	20.9					22.7
15	23.8	23.5	24.6	21.3	22.0					23.2
16	23.8	23.9	25.2	21.7	22.6					23.1
17	24.2	24.1	25.4	22.0	22.1					23.2
18	24.0	23.9	26.1	22.2	22.2					23.4
19	24.2	24.1	24.9	22.3						23.8
20	24.3	25.5	25.6	22.5						25.1

already been explained as consisting of multiples of the probable error of the difference between the averages. The curves have been drawn with the age—in weeks—plotted on the abscissa and the multiples of the probable error on the ordinates. The position of the curve above the

abscissa indicates a stimulation of growth in comparison with the series of mice that in the special case has been accepted as control, a position below the abscissa means retardation of growth. For a single curve a deviation of three to four times the probable error is desirable in order

TABLE 2

Average weight of female mice

	TEMPERATE ROOM			HOT ROOM						
		Light		Stagnant air						
				Subdued light				Light		
					Descendants					Wind
	Controls	Immigrants	Descendants	Immigrants	1 generation	2 + 3 generations	4 generations	Immigrants	Descendants	
Number weighed	34	10	27	45	36	24	11	23	24	14
age, weeks										
3	6.3	6.3	7.6	7.5	6.7	5.9	6.0	6.8	6.4	7.7
4	9.7	10.6	11.1	9.7	9.5	7.7	8.5	9.5	8.5	9.4
5	12.4	13.7	14.5	11.7	12.1	9.6	12.4	10.6	10.4	11.7
6	14.4	15.1	15.8	13.1	13.9	10 9	13.4	11.2	11.5	13.0
7	15.9	16.7	17.1	14.5	15.1	12.0	14.8	12.6	12.0	14.2
8	17.4	17.5	17.9	15.9	16.0	13.0		14.2	14.2	15.4
9	18.5	18.9	18.9	16.6	17.7	13.6		15.2	15.4	16.7
10	19.0	18.9	19.6	17.2	17.8	14.8		15.6	16.4	17.9
11	19.8	19.4	19.8	17.9	17.9	16.2		16.4	17.5	18.5
12	20.2	20.0	20.3	18.5	18.0	15.9		16.9	18.0	19.4
Number weighed	14	7	16	17	19					10
age, weeks										
13	20.6	20.6	20.9	18.4	18.3			17.1	17.9	18.3
14	21.1	20.4	20.7	18.1	18.2					19.6
15	20.9	21.0	21.7	18.7	17.8					18.5
16	21.0	21.6	21.3	18.6	19.4					19 7
17	21.6	22.2	22.1	18.4	18.1					19.4
18	21.8	22.1	22.3	18.1	17.9					19.6
19	22.4	22.7	21.3	18.5						20.1
20	22.7	22.5	22.9	20.0						20.6

to insure that very small chances exist that the observed difference is not a true one. These chances are for three times the probable error 1:21 and for four times the same error 1:142. If, however, the male and the female curves are both located on the same side of the abscissa

smaller deviations of these curves may be significant as adding a further weight to the averages.

In figure 3 the growths of the two "temperate, light" series and the "hot, stagnant air, subdued light, immigrant" series have been compared with the "temperate, subdued light" series as control. In figure 4 the "hot, stagnant air, subdued light, immigrant" series serves as a

TABLE 3

Variability of weight in male mice

AGE	TEMPERATE ROOM			HOT ROOM				
		Light		Stagnant air				
				Subdued light			Light descendants	Wind
	Controls	Immigrants	Descendants	Immigrants	Descendants			
					1 generation	2 + 3 generations		
weeks								
3	23.2	(11.7)	21.1	23.7	29.5	28.8	18.4	17.9
4	22.3	19.5	19.5	19.4	22.6	23.2	19.6	20.8
5	19.4	15.4	12.5	20.9	19.2	20.5	15.9	19.7
6	20.3	9.8	11.5	21.9	18.9	19.1	17.8	21.2
7	19.1	9.2	9.3	23.8	19.2	14.2	17.7	21.5
8	14.2	7.4	7.8	20.7	17.0	16.0	17.7	20.1
9	10.2	7.5	7.7	17.2	13.6	14.7	14.0	16.6
10	9.2	7.1	8.0	16.3	13.6	12.1	12.3	13.4
11	8.0	6.7	6.2	15.0	12.9	16.8	10.2	11.8
12	7.1	7.2	7.1	14.0	11.6			11.8
13	7.7	7.3	7.1	13.2	10.0			11.0
14	7.7	7.5	7.9	9.1	11.2			10.7
15	8.2	7.6	8.0	9.5	9.4			10.6
16	7.9	8.8	8.0	9.7	11.4			9.8
17	7.6	9.3	6.5	9.2	11.3			11.0
18	8.3	9.1	7.7	7.0	10.4			13.0
19	(4.7)	9.7	7.1	6.8				12.0
20	(4.9)	9.4	8.2	(5.7)				10.4

base line and the curves correspond to the "hot descendant" series of the first and later generations and to the "hot, wind" series. Finally, in figure 5 three pairs of various "descendant" series are compared with the corresponding "immigrant" series as base line.

The material at our disposal does not seem to leave any doubt that the radiation of light acted as a stimulant to the growth of those mice

that were exposed to light at ordinary room temperature. This applies particularly to those mice which were born in that specified environment. The difference in weight between the "light" and control series seems to diminish with increasing age. It is impossible to decide to what extent the effect of light on growth may be due to the heat rays and how much to rays of a short wave length. The possibility that, e.g.,

TABLE 4

Variability of weight in female mice

AGE	TEMPERATE ROOM			HOT ROOM					
		Light		Stagnant air					
				Subdued light			Light		Wind
	Controls	Immigronts	Descendants	Immigrants	Descendants		Immigrants	Descendants	
					1 generation	2 + 3 generations			
weeks									
3	24.3		21.7	26.3	30.0	20.3	21.0	17.5	21.7
4	20.1	12.5	18.3	22.9	24.1	16.8	21.8	15.9	24.9
5	18.1	10.3	14.8	21.4	17.8	17.0	18.1	10.9	24.9
6	16.2	10.5	11.4	20.6	17.6	17.1	13.5	13.3	28.3
7	13.3	6.3	10.8	18.1	15.3	18.5	18.3	14.3	28.6
8	12.6	6.5	8.7	16.7	14.5	18.4	14.4	11.4	25.2
9	11.4	8.9	9.3	16.2	13.0	18.5	12.5	9.7	19.3
10	11.4	7.0	9.0	15.2	11.9	18.7	15.1	7.7	17.6
11	10.1	7.5	7.4	15.8	13.1	17.2	13.2	8.6	16.0
12	10.4	7.7	7.5	15.7	13.2	17.0	13.4	7.7	15.6
13	10.6	7.1	7.3	14.4	14.0		12.0	7.7	(21.2)
14	9.4	7.5	8.8	(21.2)	14.1				11.7
15	10.9	6.9	7.1	15.2	12.4				18.2
16	10.1	6.6	7.4	15.4	11.9				8.5
17	7.4	6.8	5.0	16.3	12.2				9.5
18	7.4	7.0	8.6	15.4	10.6				10 2
19	7.8		5.6	16.7					10.5
20	(9.3)		9.4	15.9					10.9

a greater amount of exercise of the "light" animals may have contributed to the stimulation of growth can be excluded. It was found that these mice rested for at least as long periods of time as the controls.

The physical development of mice that, when 3 weeks old, were transferred to the "tropical" room and there kept in a stagnant hot atmosphere, was uniformly retarded. These experimental results are

in full agreement with what might have been expected. Mice that are born in the hot environment differ from the "immigrants" in that their growth curve in its earlier part is at a considerably higher level and

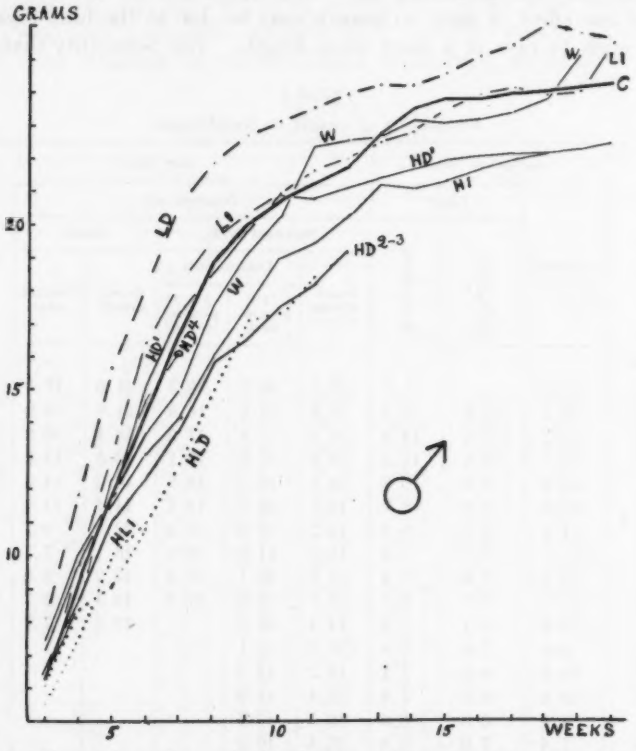

Fig. 1. Comparison of growth of male mice. Average weights. C = Controls; LI = light, immigrants; LD = light, descendants; HI = heat, stagnant air, subdued light, immigrants; HD^1 = heat, stagnant air, subdued light, descendants, 1st generation; HD^{2+3} = heat, stagnant air, subdued light, descendants, 2nd and 3rd generation; HD^4 = heat, stagnant air, subdued light, descendants, 4th generation; W = heat, wind; HLI = heat, stagnant air, light, immigrants; HLD = heat, stagnant air, light, descendants.

coincides with the control curve at least as far as the males are concerned. At a more advanced age the "descendants" seem to cease to grow until their curve coincides with the "immigrant" curve.

In the next two generations of mice born in the hot room we miss the initial stage of comparatively rapid growth and the whole curve remains low, especially for the females. This would possibly indicate that in these generations the unfavorable effect of the new environ-

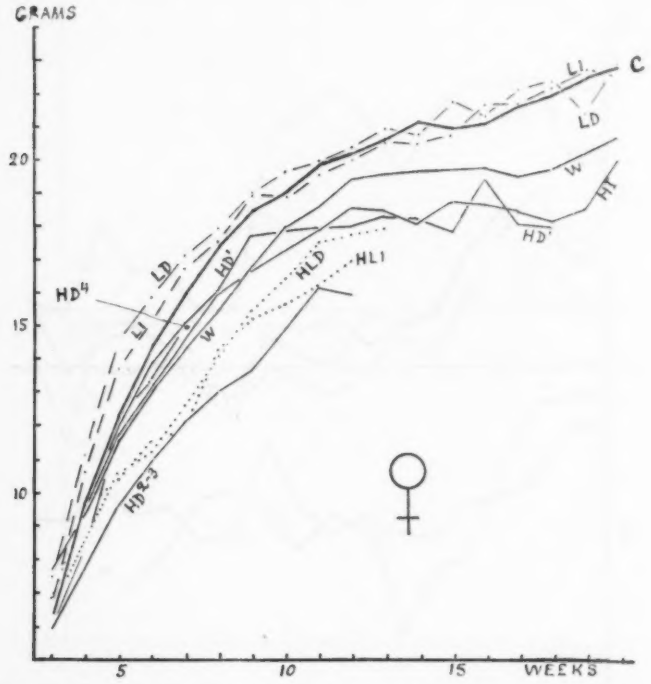

Fig. 2. Comparison of growth curves of female mice. Average weights. *C* = Controls; *LI* = light, immigrants; *LD* = light, descendants; *HI* = heat, stagnant air, subdued light, immigrants; HD^1 = heat, stagnant air, subdued light, descendants, 1st generation; HD^{2+3} = heat, stagnant air, subdued light, descendants, 2nd and 3rd generations; HD^4 = heat, stagnant air, subdued light, descendants, 4th generation; W^4 = heat, wind; *HLI* = heat, stagnant air, light, immigrants; *HLD* = heat, stagnant air, light, descendants.

ment becomes manifest. It is regrettable that the study of the fourth generation was continued only until the seventh week. The considerable rise in the growth curve of this generation may signify that at this point of racial adaptation the adjustment of the energy balance

had been taken over by other efficient mechanisms so as to allow the growth to proceed at a velocity that approaches the normal.

We have seen that radiation of light stimulates physical development when the environmental conditions otherwise are favorable for a normal

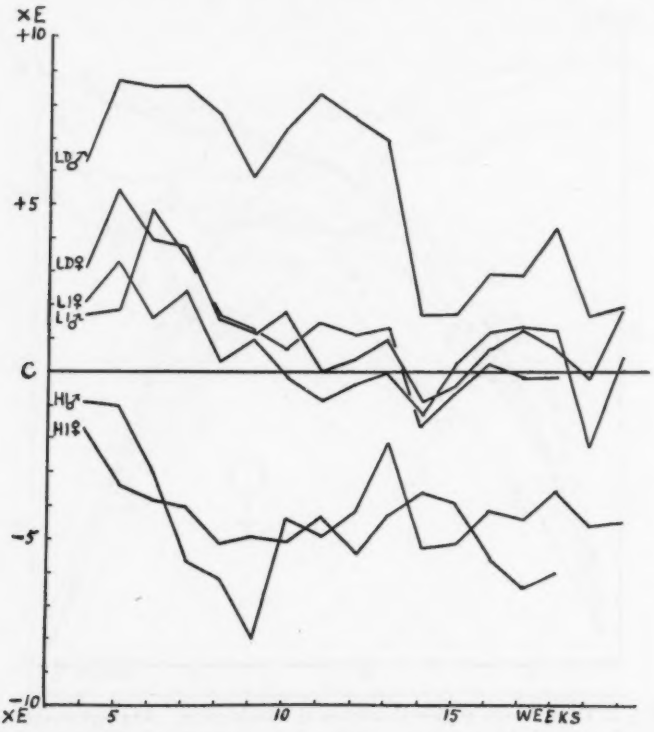

Fig. 3. Comparison of growth curves of "light" and "heat, immigrant" mice with control mice. Multiples of probable error of difference, E, between average weights. C = Controls; LI ♂ = light, immigrants, males; LI ♀ = light, immigrants, females; LD♂ = light, descendants, males; LD ♀ = light, descendants, females; HI ♂ = heat, stagnant air, subdued light, immigrants, males; HD ♀ = heat, stagnant air, subdued light, immigrants, females.

output of heat. When these conditions are not present, e.g., when the animals are confined to a hot room with stagnant air the disproportion between heat production and heat output becomes so great that

the organism reacts with retardation of growth in a degree that exceeds the retardation in heat without the addition of light.

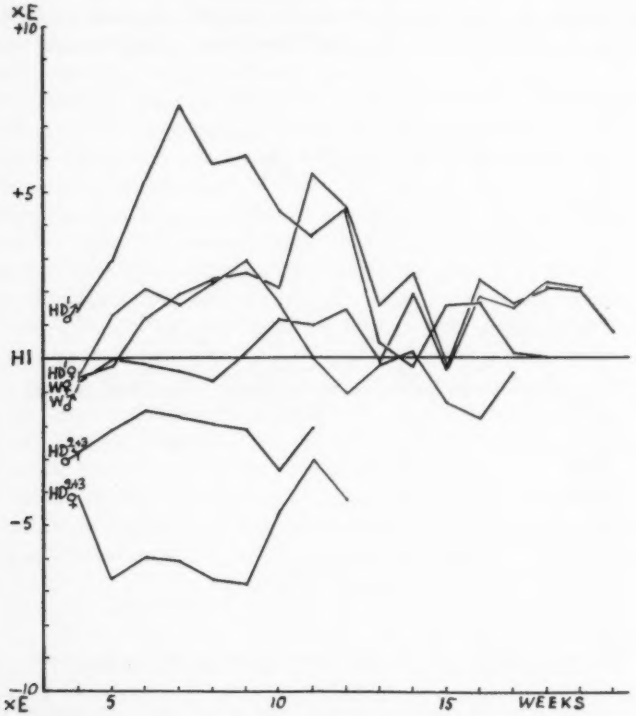

Fig. 4. Comparison of growth curves of "heat, stagnant air, descendants" and "wind" mice with "heat, immigrant" mice. Multiples of probable error of difference, E, between average weights. HI = heat, stagnant air, subdued light, immigrants; $HD^1♂$ = heat, stagnant air, subdued light, descendants, 1st generation, males; $HD^1♀$ = heat, stagnent air, subdued light, descendants, 1st generation, females; $HD^{2+3}♂$ = heat, stagnant air, subdued light, descendants, 2nd and 3rd generations, males; $HD^{2+3}♀$ = heat, stagnant air, subdued light, descendants, 2nd and 3rd generations, females; $W♂$ = heat, wind, males; $W♀$ = heat, wind, females.

It has repeatedly been found that, when the air is in motion, the overheating of the body which otherwise would occur in humid heat, is prevented. The effect of wind on growth has, as far as I know, not

been subjected to experimental investigation. My results in this respect point to a favorable influence. Although my material is rather limited and the "wind" curves in figure 4, therefore, do not deviate as much from the base line as would be desirable in order to definitely settle the problem, the fact that both the "male" and "female" curves

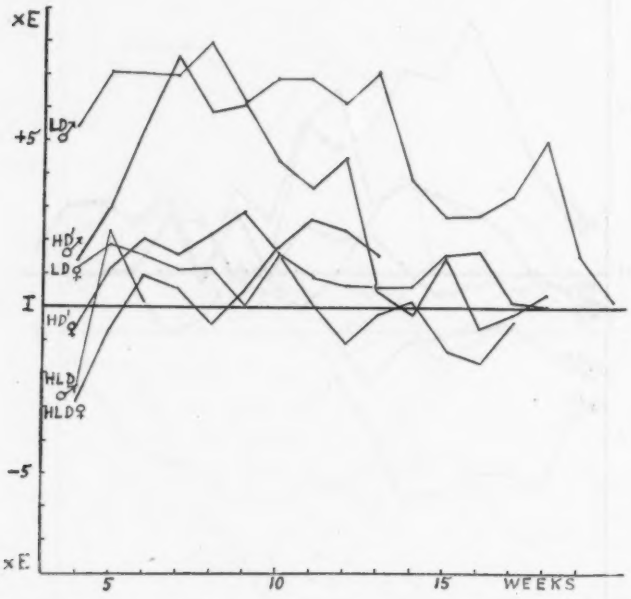

Fig. 5. Comparison of growth curves of "immigrant" and "descendant" mice in same environment. Multiples of probable error of difference, E, between average weights. I = Immigrant mice; LD ♂ = light, descendants, males; LD ♀ = light, descendants, females; HD^1 ♂ = heat, stagnant air, subdued light, descendants, 1st generation, males; HD^1 ♀ = heat, stagnant air, subdued light, descendants, 1st generation, females; HLD ♂ = heat, stagnant air, light, descendants, males; HLD ♀ = heat, stagnant air, light, descendants, females.

are located on the plus side justifies us in assuming that circulation of air may play an important rôle in promoting the well-being of growing animals. In the interest of the physiological hygiene of the Tropics this phase of the subject deserves a thorough re-investigation.

It is a fact well known to students of the racial biology of white settlers in tropical countries that women are liable to suffer more

from the heat than men do. Although hygienic and social conditions may contribute to this difference in the power of adaptation between the sexes, the possibility is not excluded that the difference might have a biological significance. The fact that the female mice in my series reacted to the humid heat by a far greater retardation of growth and also exhibited less resistance in other respects to the new environment, deserves therefore to be mentioned. While the females seem to be more susceptible to agencies retarding growth, the male sex is apparently more easily affected by factors that neutralize the unfavorable environment.

Sufficient material seems to be at our disposal to disclose a marked difference in growth between mice that were transferred to a new environment when in a growing state, and mice that were born in the same environment from "immigrant" parents. This applies to climatic environments characterized by humid heat, radiant energy or both. A glance at figures 1, 2 and 5 will elicit this remarkable fact. In such of the three environments in which "immigrants" and "descendants" are compared the growth of the latter is more rapid. The degree of this difference between the generations seems to be proportional to the favorability of the environment.

Robertson (14) has published data that indicate that the retardation of growth that occurs in children as a result of an unfavorable environment is accompanied by a low variability of weight. This rule does not apply to our present study with exception possibly of the female mice that were born in the hot room and from birth exposed to strong light. In all the other hot room series the variability of weight is uniformly high. It is probable that this phenomenon may be the criterion of the existence of opposing climatic factors, some accelerators and others depressors of growth. When one of these groups of factors gains the upper hand the variability of weight seems to drop. The low variability of weight of mice that in the temperate room were exposed to light forms an illustration of the effect of the addition of a single factor acting as a growth stimulant.

My data concerning the fertility of the mice in the hot environment are limited. Since they give an idea of the relative fertility in several succeeding generations they deserve, however, to be reported. The total number of young born to twelve females that were transferred to the hot room in a pregnant state was 63. The average size of these litters is consequently 5.25. The average size of seven litters in the third generation of these mice was 5.57 and of eight litters in the fourth

generation 5.50. The average size of these 15 litters is 5.53. Robertson (15) gives 5.15 as the average of 241 litters of the same stock at ordinary room temperature. The average size of 50 litters I observed in the same climatic environment was six. These figures indicate that, notwithstanding the inbreeding, a slowly progressing increase of fertility had taken place. They further show that the heat mice had participated in this increase. Even considering the small material, we may say that at least no decrease of fertility had taken place. Steinach and Kammerer (3), from experiments on the effect of heat on the fertility of rats, report the average size of a litter at 20°C. as ten, at 25° as eleven and at 30° as eight. I am inclined to the view that the decrease of fertility in these experiments was due to the abnormally high normal fertility. My results indicate that when the fertility of the parent generation is of medium dimensions, a species may be capable of preserving this fertility uninfluenced by a hot environment.

SUMMARY

1. Exposure to artificial light at ordinary room temperature accelerates the growth of mice.

2. Confinement in a stagnant hot and humid atmosphere retards the growth of mice that were transferred to the new climatic environment immediately after separation from their mothers.

3. Succeeding generations of mice born in a hot and humid environment behave differently in their reaction to this environment. The first native generation may develop normally. The two next generations may elicit the greatest effect of the hot climate, at least as far as their growth is concerned. It is suggested that a racial adaptation may finally occur.

4. Exposure to artificial light in humid heat adds to the retarding effect on growth produced by the latter climatic factor.

5. Circulation of the hot and humid air neutralizes partly the unfavorability of the tropical environment for the growth of animals.

6. The growth of male mice is less retarded by an unfavorable environment and more accelerated by growth stimulating climatic fac torsthan the growth of female mice.

7. The growth of mice that are born in a new environment but the intrauterine development of which falls partly outside this environment grow faster than animals that are transferred to the same environment when in a growing state.

8. It is suggested that the higher variability of weight that occurs in an artificially produced tropical climate may depend upon the operation of opposing climatic factors of which one group may act as growth accelerators, the other as depressors of growth.

9. When the fertility of a mice colony is normal, it is not necessarily diminished by confinement of the mouse in humid heat for several generations.

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STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

II. Relations of the Body Form and Especially the Surface Area to the Reactions Released by and the Resistance to a Tropical Climate

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In the previous paper (1) I have suggested that the retardation of growth in a tropical climate may assist in promoting the cooling of the body by increasing the relative body surface. In this paper we will turn our attention to the possibility of an independent reaction of the peripheral parts of the body to the heat, which may be instrumental in changing the body surface:weight ratio in a direction favorable for the maintenance of the energy balance.

Sumner (2), in extensive investigations on mice, directed to establishing the inheritance of acquired characters, discovered an increase in length of tail, ears and feet in his hot room animals. This increase could be demonstrated also in mice that were born in the cold room of mothers which had lived in the hot environment. Sumner incidentally mentions the possibility that these changes might have some physiological significance but he makes no attempt to specify the advantage the animals may receive from them.

Rubner (3), in his work on the functions of the body surface in determining the basal metabolism, gives the surface:weight ratio of mice as 2.30. He has apparently left the area of the ears out of his calculations. It is evident, however, that Rubner, while assuming that the ears did not participate in the heat output in a cool environment, was aware of the fact that the ears—and also the tail—may serve as auxiliary cooling organs in a hot climate. I have found numerous occasions to confirm this hypothesis. The ears and the tail were in my hot room animals greatly hyperemic. Especially when the room temperature was allowed to rise to a height close to the body temperature, these external

parts were bright red. To the heat radiating organs I am also inclined to add the scrotum of male mice. An enlargment of the scrotum has repeatedly been reported in rats and mice that have been exposed to high external temperature. My heat mice formed no exception from this rule. In a rat colony that I have afterwards had opportunity to observe in actual Tropics every male had a hanging scrotum. It is obvious that such a sac of the integument, devoid of hair as it is, must offer for the animals an ideal cooling apparatus.

Considerations of this kind, naturally, do not find any human application. The possibility is, however, not excluded that even man may possess some means of increasing his surface : weight ratio. Since surface area as known is a function of height as well as of weight, an increase in the height : weight ratio will also result in an increase in the surface : weight ratio. The slender type of the aboriginals in the Tropics—with exception of Polynesians—may serve to illustrate this point. Observations in Java (4) on the height and weight of Dutch children have revealed that the height : weight ratio increases considerably faster in Java than in Europe. We may therefore infer that the surface : weight ratio for children of the same weight is higher in the former place.

My experiments to determine whether the surface : weight ratio undergoes any change in heat, when animals of the same weight are compared, were performed on two batches of mice, in which a number of litters were equally represented and of which one was kept in the temperate room until two months old and the other in the hot room up to the same age. Other environmental conditions were identical. Immediately after being killed, their weight and length were recorded, the latter measured without stretching from the tip of the nose to the tail end. The tail was measured from anus. One of the ears was cut out and trimmed along the protruding fold. It was then pasted on a piece of millimeter paper and the area computed. Tail and feet were cut from the carcass and their total skin area determined. The rest of the skin was finally carefully removed and spread out without unduly stretching on plate glass. A piece of millimeter paper was laid on top of the skin and with the empty side of the glass turned toward a window the contours of the skin were drawn as accurately as possible. The sum of the area of the skin plus the area of the ears—multiplied by 4—plus the area of tail and feet was taken as representing the total body surface.

Tables 1 and 2 contain data—for males and females separately—regarding body weight, total body surface, the constant in Meeh's surface

TABLE 1

Body weight, body surface, the constant in Meeh's surface formula, surface : weight coefficient, ear surface, ear surface in per cent of total surface, body length, length : weight ratio, tail length and tail length in per cent of total length in skinned male mice

NUMBER	BODY WEIGHT	BODY SURFACE	k IN $k\sqrt[3]{W^2}$	SURFACE : WEIGHT	EAR SURFACE	EAR : BODY SURFACE	BODY LENGTH	LENGTH : WEIGHT	TAIL LENGTH	TAIL : BODY LENGTH
Controls										
	grams	*sq. cm.*		*sq. cm.*	*sq. cm.*	*per cent*	*cm.*	*cm.*	*cm.*	*per cent*
1	26.5	90.4	10.2	3.41	4.40	4.9	9.8	0.37	6.8	41.0
2	26.4	87.6	9.9	3.32	4.12	4.7	9.7	0.37	6.9	41.6
3	25.3	97.9	11.4	3.87	3.88	4.0	9.5	0.38	7.6	44.4
4	24.6	90.6	11.7	3.68	4.08	4.5	9.5	0.39	6.5	40.6
5	24.2	81.6	9.7	3.37	4.88	6.0	9.5	0.39	6.6	41.0
6	23.3	88.2	10.8	3.79	4.36	4.9	9.5	0.41	6.5	40.6
7	22.2	83.3	10.5	3.75	3.60	4.3	9.3	0.42	6.0	39.2
8	21.4	83.8	10.9	3.92	3.40	4.1	9.2	0.43	6.1	39.9
9	21.2	82.1	10.7	3.87	3.68	4.0	9.2	0.43	6.2	40.3
10	21.2	87.7	11.4	4.14	3.68	4.2	9.4	0.44	6.8	42.0
11	20.8	80.8	10.7	3.88	3.12	3.9	9.1	0.44	6.9	43.1
12	20.6	80.4	10.7	3.90	4.28	5.3	9.1	0.44	7.3	44.5
13	20.1	77.6	10.5	3.86	3.40	4.4	9.0	0.45	5.5	38.0
14	19.8	78.0	10.7	3.94	3.56	4.6	8.8	0.44	7.2	45.0
15	19.0	78.0	10.9	4.10	3.60	4.6	9.0	0.47	5.9	39.6
16	18.6	75.3	11.7	4.05	3.72	4.9	8.7	0.47	5.8	40.0
Average....	22.2	84.0	10.6	3.78	3.84	4.6	9.3	0.42	6.5	41.3
Heat mice										
1	23.4	84.0	10.3	3.59	4.88	5.8	9.5	0.41	6.8	41.7
2	20.3	74.3	10.0	3.66	4.64	6.3	9.0	0.44	7.3	44.8
3	20.0	77.5	10.5	3.88	4.12	5.3	9.0	0.45	6.9	43.4
4	19.5	74.7	10.3	3.83	3.92	5.3	9.0	0.46	6.4	41.6
5	19.0	70.9	10.0	3.73	4.60	6.5	8.7	0.46	7.2	45.3
6	19.0	84.7	11.9	4.46	4.48	5.3	8.7	0.46	7.4	46.0
7	18.2	75.5	10.9	4.15	3.32	4.4	8.7	0.48	7.2	45.3
8	18.2	79.5	11.5	4.37	4.24	5.3	8.8	0.48	6.8	43.6
9	16.6	79.6	12.2	4.80	3.88	4.9	8.4	0.51	6.6	44.0
10	16.4	75.5	11.7	4.61	4.28	5.7	8.5	0.52	7.0	45.2
11	15.8	74.0	11.8	4.68	3.40	4.6	8.0	0.51	5.5	40.8
12	15.6	73.5	11.8	4.71	3.72	5.1	8.3	0.53	6.8	45.0
13	15.4	77.1	12.5	5.01	4.48	5.9	8.1	0.53	7.1	46.7
14	14.2	68.0	11.6	4.79	3.64	5.4	8.0	0.56	6.3	44.1
Average....	18.0	76.6	11.2	4.26	4.12	5.4	8.6	0.48	6.8	44.1

TABLE 2

Body weight, body surface, the constant in Meeh's surface formula, surface : weight coefficient, ear surface, ear surface in per cent of total surface, body length, length : weight ratio, tail length and tail length in per cent of total length in skinned female mice

NUMBER	BODY WEIGHT	BODY SURFACE	k IN $k\sqrt[3]{W^2}$.	SURFACE WEIGHT	EAR SURFACE	EAR : BODY SURFACE	BODY LENGTH	LENGTH : WEIGHT	TAIL LENGTH	TAIL : BODY LENGTH
Controls										
	grams	*sq. cm.*		*sq. cm.*	*sq. cm.*	*per cent*	*cm.*	*cm.*	*cm.*	*per cent*
1	21.3	71.4	9.3	3.35	3.56	5.0	9.0	0.42	6.8	43.0
2	20.2	74.1	10.0	3.67	3.84	5.2	9.0	0.45	6.0	40.0
3	19.3	71.9	10.0	3.73	4.36	6.1	8.8	0.46	7.0	44.3
4	18.9	68.3	9.6	3.61	3.64	5.3	9.0	0.48	6.2	40.8
5	18.6	71.8	10.2	3.86	3.76	5.2	8.5	0.46	6.5	43.3
6	18.6	74.0	10.5	3.98	2.96	4.0	8.7	0.47	6.4	42.4
7	17.7	72.8	10.2	4.11	3.76	5.2	8.8	0.50	6.3	41.7
8	16.6	69.3	10.6	4.17	3.80	5.5	8.8	0.53	6.0	40.6
9	15.9	62.4	9.9	3.92	3.72	6.0	8.3	0.52	5.7	40.7
Average....	18.6	70.7	10.1	3.80	3.72	5.3	8.8	0.47	6.3	41.7
Heat mice										
1	23.7	84.2	10.2	3.55	4.72	5.6	9.1	0.38	8.0	46.8
2	17.7	76.9	11.3	4.34	4.92	6.4	8.5	0.48	7.1	45.5
3	17.4	67.5	10.0	3.88	3.92	5.8	8.7	0.50	6.7	43.5
4	17.0	70.9	10.7	4.17	3.92	5.5	8.5	0.50	6.9	44.8
5	17.0	71.0	10.7	4.18	4.24	6.0	8.4	0.49	6.5	43.6
6	16.9	70.3	10.7	4.16	4.60	6.5	8.6	0.51	6.0	41.1
7	16.6	70.8	10.9	4.27	4.28	6.0	9.0	0.54	6.5	42.0
8	16.5	69.3	10.7	4.20	3.96	5.7	8.0	0.48	6.4	44.5
9	15.0	65.5	10.8	4.37	4.08	6.2	8.0	0.53	6.2	43.7
10	15.0	66.8	11.0	4.45	4.16	6.2	8.1	0.54	6.8	45.6
11	14.9	66.4	11.0	4.46	4.04	6.1	8.3	0.56	6.2	42.8
12	14.3	68.0	11.5	4.76	4.20	6.2	8.0	0.56	7.0	46.7
13	14.1	63.2	10.8	4.48	3.84	6.1	8.4	0.60	5.8	40.8
Average....	16.6	70.1	10.8	4.21	4.24	6.0	8.4	0.51	6.6	44.7

formula, surface : weight coefficient, ear surface, ear surface in per cent of total surface, body length, length : weight ratio, tail length and the tail length in per cent of body length.

In conformity with the results of our growth studies we find a retardation of growth in the heat mice, which in this series in average amounts

to 4 grams for the males and to 2 grams for the females. The decrease of the average body surface is 9 per cent for the male and less than 1 per cent for the female sex. Calculated from Meeh's formula, body surface = a constant × cube root of squared body weight, a diminution of body surface, accompanying the decrease in weight, would have occurred, which in the male mice would have amounted to 15 and in the female mice to 8 per cent. This, supposing the same constant would be valid for both series of mice, which would be true if no change in body form had occurred in the heat mice. In Meeh's formula the constant is, as known, an expression of the body form and varies with different animals and to some extent also in the same species. We know that in Du Bois' formula for calculating the surface area of man the height has also been considered. For animals no such formula is available.

Rubner (3) has calculated the constant in Meeh's formula for the white mouse as 11.4. He found that after 4 days' starvation the factor rose to 12.3. We possess in this study all the necessary data for calculating the constant in both series of mice. For the male controls it varies between 9.7 and 11.7 and is in average 10.6. Corresponding figures for the heat male mice are 10.0, 12.5 and 11.2. The range for the control female mice is 9.3 to 10.6 with 10.1 as mean and for the heat female mice 10.0 to 11.5 with 10.8 as average. It is evident that in the heat mice the body form is instrumental in rendering available a larger cooling surface and that the male mice in both control and heat series are better qualified for cooling their bodies than are the female mice.

Regarding the relation of total superficial area to body weight it is seen that a larger skin area is available for each unit of body weight in the heat mice than in the temperate series. In the male control series the surface: weight coefficient varies between 3.32 and 4.14 and is in average 3.78. In the male hot room series the figures are 3.59, 5.01 and 4.26. In the female control series the mean is 3.80 with a range from 3.35 to 4.17, while the average in the hot room series for the same sex is 4.21 with a maximum of 4.76 and a minimum of 3.55. Considering averages we may say that an increase of cooling area had taken place in the "tropical" room that amounts in the male mouse to 12 and in the female mouse to 11 per cent. Since Moulton (5) has shown that body weight, surface area and amount of living protoplasm —as estimated from the nitrogen content—are functions of each other, we may assert that each gram of living substance in the hot environment has a larger cooling area at its disposal.

Since we know that a decrease in weight is accompanied by a relatively larger body surface it remains to consider whether the retardation of growth in the animals, which had been exposed to the hot environment, could, eventually be the sole cause of this phenomenon. That this is not true and that besides factors associated with the body form are responsible for the change, has already been made probable in our discussion of the constant in Meeh's formula. We may find further corroboration for this conception from a consideration of the size of the external parts of the body, viz., the size of the ears and the length of the tail. We find that the ear surface, in proportion to total body surface, has increased in the male mice from 4.6 to 5.4 per cent. In the female mice the relative increase of the ear surface is from 5.3 to 6.0 per cent. The length of the tail in relation to body length has increased from 41.3 to 44.1 per cent in male mice and from 41.7 to 44.7 per cent in female mice. Furthermore, the length : weight ratio has increased from 0.42 to 0.48 in males and from 0.48 to 0.51 in females. These results indicate that, irrespective of the change in body weight, morphological changes have taken place in peripheral parts of the body of the heat animals with a resulting enlargement of the body surface. In order to further confirm this conception I have in figure 1 plotted the surface : weight coefficients as ordinates, the abscissa giving the age in weeks.

As only a few individuals of lower body weight were available in the control series I have added half a dozen one week younger mice for comparison. With exception of a few cases—the probably rather large experimental error of the method must be considered—the control figures group themselves round almost a straight line. The curve for the heat mice again, while for higher ages at a low level, makes in direction of the younger mice a sharp bend upward indicating in these mice a high surface : weight coefficient. We find, consequently, that the retardation of growth in the heat mice has gone hand in hand with morphological changes of peripheral parts of the body which both ultimately result in supplying the organism with a larger cooling area per unit body weight.

It now remains to discuss the possibility whether an enhancement of the resistance toward heat actually occurs in those mice which have acquired the modified body surface. A convenient method of testing this point seems to consist in exposing animals that have had time to adapt themselves to moderate heat to excessive temperatures. My first experiment of this kind was an involuntary one. In a preliminary

hot room series, consisting of 23 mice, an accident allowed the temperature of the room to rise to 39° for one night two months after the commencement of the series. Next morning I found only four survivors in the lot, of which three were males. These were all individuals which had undergone a retarded growth. Figure 2 clearly demonstrates

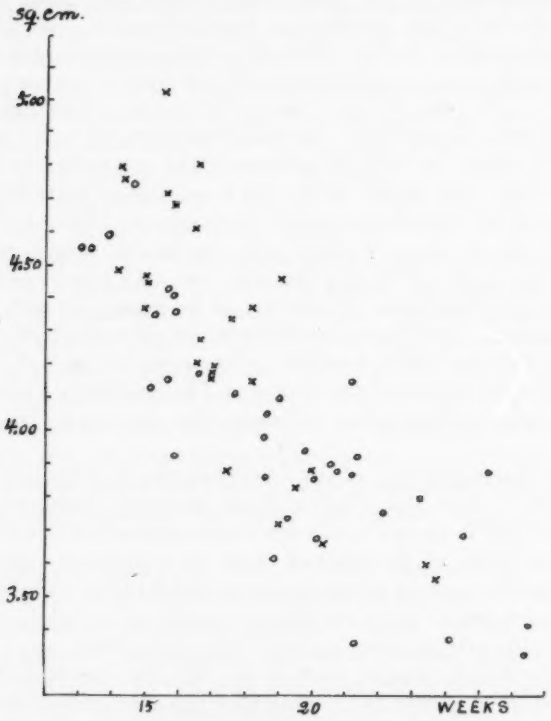

Fig. 1. Surface: weight coefficients and age. O = controls; X = heat mice.

the correlation of growth velocity and chance of survival in excessive heat.

In another experiment I tested the relative resistance to an excess of light—the heating effect of which was probably the primary factor—in two series of mice one of which had been bred in the hot room in subdued light, the other exposed to the light from a 100 watt "Mazda." The source of light in the experiment consisted of two 100 and two

60 watt "Mazda" globes, suspended about 40 cm. above the bottom of the cages. The air temperature was 33°, the relative humidity 60 per cent. A thermometer, the bulb of which was buried in the sawdust registered 38–39°. After 4 hours of exposure one third of the animals that had previously been protected from light survived the test. The proportion among the animals which had been accustomed to light was three in ten. One animal succumbed in the former batch

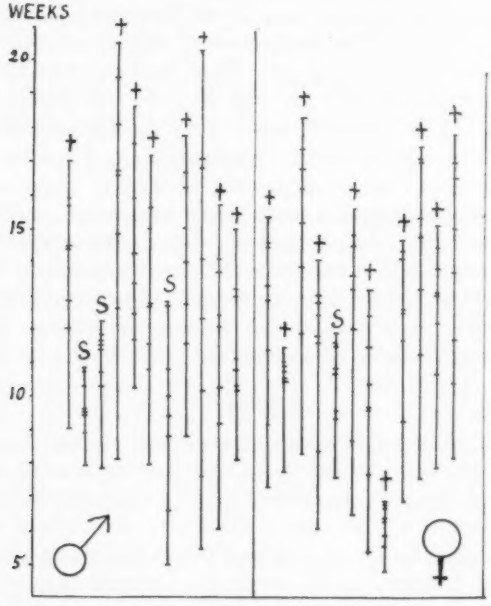

Fig. 2. Heat resistance experiment. Each of the 23 mice represented by a vertical growth line. *S* = survived; + = dead.

after only one hour of exposure. The other deaths occurred at intervals which were about the same in both cages. In the beginning of the experiment the animals drank freely of the water offered them and their movements were coördinated, later they ran around in a dazed condition. Apparently those mice that were most active succumbed quickest. The resistance did not seem in this case to be correlated by body weight. My impression that the central nervous system was primarily affected, that the deaths were due to real "sunstrokes" was supported

by post-mortem examinations. The meninges were highly edematous. The brain substance and the meninges showed numerous hemorrhages. Other typical signs of a "sunstroke" were also present.

A third heat resistance experiment in which I hoped to gain some information about the relative resistance of mice, which were adapted to different environments, to hot air, dry bulb = 30° of high humidity—near the saturation point—did not yield any conclusive result other than that mice succumb quickly in such a climatic environment. A short time after the commencement of the experiment the animals became covered with profuse perspiration and they all died at approximately the same time in all series. When the mice were weighed after death it was found that they had lost up to 10 per cent of their body weight in their futile efforts to affect body cooling by evaporation of water in the humid atmosphere. Sweating seemed in mice to be only an ultimate refuge in attaining the output of heat. Even at temperatures that were only slightly below body temperature animals which were adapted to heat were completely dry. Other individuals, however, which only for a shorter time had been exposed to the heat or which had been accustomed to the draught from a revolving fan were perspiring under these exceptional climatic conditions. If the temperature was not immediately lowered they soon succumbed.

SUMMARY

1. Retardation of growth may in a tropical climate assist in combating the overheating of the body. This effect is suggested as due to the enlargement of body surface area that accompanies the lower body weight.

2. Animals may be able by certain modifications of morphological characters, viz., enlargement of peripheral body parts, to further improve their cooling facilities.

3. A few experiments seem to indicate that, while a certain resistance to higher temperatures may be acquired by animals which have been adapted to external heat, this does not apply to environment in which light and humidity are the predominating climatic factors.

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STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

III. Effect of the Tropical Climate on Growth and Pigmentation of Hair and the Dependence of these Integumental Functions on the Temperature Coefficient Law

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In the previous paper (1) we have discussed some modifications which certain peripheral parts of mice undergo in a hot environment. The physiological usefulness of the enlargement of ears and scrotum and the elongation of the tail to the animals seems to be well established. The physiological factors that are at play in the development of these modifications are still obscure. The increase of blood flow through the integumental capillaries is probably one of these factors. Some evidence I have collected along other lines seems, however, to indicate that different histological components of the skin may be dependent in their proper functioning on different temperature coefficients. It is therefore possible that the extent of cellular activity that determines the size of above-mentioned peripheral parts may be a function of the external temperature. Although, naturally, the sequence of these events must largely escape direct observation, we will in the following find occasion to strengthen the hypothesis. Our attention in this paper will be chiefly directed to elucidating the rôle that temperature may play in hair growth and pigment production. By analogy the importance of the temperature coefficient law for the development of other integumental appendices will receive some support.

Rubner (2) has shown that the amount of hair is closely correlated to the effectiveness of the heat regulation of the body. We may therefore infer that in a tropical climate where demands are made upon an efficient cooling power of the skin the fur of animals should become thinner. This has actually been found to occur. Wild animal species extending over large areas from north to south have a thinner coat in the

southern part of their habitat (3). It is said that thoroughbred sheep in hot climates do not yield the same amount of wool as in the temperate parts of the same continent. Sumner (4) has experimentally verified this phenomenon. The total amount of hair he shaved from his cold room mice was consistently heavier than what he got from those mice which were adapted to a hot environment.

In connection with my measurements of the surface area of mice, reported in the previous paper, I found opportunity to compare the hair growth in two series of a genetically homogeneous mouse colony, one of which was kept at ordinary room temperature, the other transferred, when 3 weeks old, to a hot and humid environment. The mice were skinned at an age of 10 weeks and the total amount of hair removed with a safety razor. The average weight of hair of the control animals was 364 mgm. and of the heat mice 372 mgm. These figures indicate that the response of the mouse to external heat by thinning its fur may not be immediate. It must, however, be remembered that since the relative amount of moulting is impossible to determine, the experimental error in estimations of this sort must be rather large. It must further be borne in mind that in Sumner's experiments the control mice were exposed to considerably lower temperatures than was the case in my series.

In order to further test the promptitude of the effect of temperature changes on hair growth I have supplemented my mouse studies on this point with observations on the hair growth of a man for three periods during an eight months' stay in the Tropics, the first period commencing 2 months after arrival. The firmness of the hair of this subject made him especially suited for a study of this kind. The hair was at not too frequent intervals cut with machine to a standard length of 1 cm. During the first period—middle of December to middle of March—which in tropical Australia is characterized by a temperature round 30°C. and a relative humidity seldom below 80 per cent, the average daily hair growth was 161 mgm. During the next period—middle of March to middle of June—when the atmosphere was cooling off, the growth was 168 and during two winter months 176 mgm. The average temperature in winter was 20° with relatively low humidity. These results seem to indicate a dependence of hair growth on temperature, the maximum growth taking place inside the temperate range. The growth of nails was also observed in the same subject. In summer the average daily nail growth was 14.6 and in the cooler season 12.6 mgm. Could this stimulation of the growth of

finger and toe nails at high temperature be a parallel to the phenomena we referred to in the introduction of this paper?

I regret that I did not find time to repeat my weighings of the amount of hair in those generations of my mouse colony that were born in the hot environment. My impression was that the fur of the animals became progressively thinner, although even in later generations individuals were found that had a fluffy coat. The majority of mice in the fourth generation were almost hairless. Although we must not lose sight of the possibility that genetical factors may have coöperated in producing these skinny looking creatures—which nevertheless exhibited an almost normal velocity of growth—I am inclined to the view that environmental factors were chiefly responsible for their appearance. I find support for this view from the fact that about 2 weeks after the transfer of the same mice to a cool environment, they had acquired a fur that almost resembled a normal one. This tends to confirm our previous conclusion that the optimal temperature for hair growth is exceeded in a tropical climate.

In our inquiry into the temperature coefficients of different skin functions we now turn our attention to the effect of heat on pigmentation. An array of facts, anthropological and zoölogical, indicates that the intensity of integumental pigmentation may depend on climatic factors. The popular belief is that the intense solar radiation and primarily the actinic sun rays are the principal agents in producing the deeper coloration of animal species toward the equator. A number of experimental investigations has nevertheless been reported which point to an independent effect of increased temperature on pigmentation. Experiments on insects are in this respect somewhat conflicting, the best controlled investigations, however, indicating a darkening of color, in heat (5). Uhlenhuth (6), in his work on metamorphosis and pigmentation of batrachia, found that he could, by lowering or increasing the environmental temperature cause the specific coloring of Amblystoma tigrinum to disappear or become manifest. Bonhote (7) kept rodents of the species Meriones in humid heat for a few weeks, apparently protected from light, and observed a darkening of their coat color.

The coat color of mice has, as known, been a favorite field of research for geneticians. The conception of Cuénot (8) that at least two "factors" are necessary for color production, one representing the "chromogen" and the other the "zymogen," has considerably stimulated investigations along these lines. The conception was, however, a

qualitative one—presence or complete absence—and to conform with facts it was found necessary to introduce a number of color modifying factors. Cuénot explained albinism as a result of absence of either the chromogen or enzyme or both. Durham (9) concluded from experiments which have not been confirmed, that there may be present, in the skin of rabbits and rats, three specific enzymes for production of the black, yellow and chocolate color. She believed that these enzymes are lacking in albinos. Von Fürth's (10) suggestion that melanin is produced by the action of tyrosinase on tyrosin has given impetus to biochemical studies along these lines. Gortner (11) discovered that tyrosinase may be inhibited in its action by certain phenolic compounds and he thinks that such an inhibition is the underlying cause of the production of "dominant whites." Onslow (12) has confirmed this statement and found further that the pigmentation of an animal is a quantitative one, the same melanin giving all the shades from light yellow to black. The same investigator was unable to recover any tyrosinase from the macerated skins of recessive albinos but found that phenolases were present in appreciable amounts. It is noteworthy and gives rise to the suspicion that her extraction method, although it appears to be faultless, may not have been so, to hear that Onslow was also unable to obtain any tyrosinase from the skins of light yellow strains.

Riddle (13) has severely criticised the explanations geneticians give about the causes of albinism. He thinks that it is absurd to speak about gametes in which either the chromogen or enzyme is absent. I cite a few lines of his paper: "There can scarcely be any doubt that certain regions, owing to new structure, *new environment, new conditions* are able to oxidize different protein substances with variable ease and to a variable extent and even in a different way." And further: "The specific color of an animal is an index not of the processes in the germ from which this animal arose, of certain chromogens and specific zymogens, and the absence of wide series of others, but this specific color means that a process with a wide range of possibilities, because of a *particular physiological state and environmental conditions* has struck this particular equilibrium. *One and the same organism has within it all that is necessary to move the equilibrium up and down.*" The words in this citation that have important bearing on our present problem are italicized by me.

After this somewhat cursory review of a research field that still remains largely in an uncultivated state, I will proceed to report certain

facts with bearing on the pigmentation problem, which came to my attention during the course of my acclimatization studies. My material for these studies consisted of white mice that for a great number of generations had been found, by trained observers, to possess all the requirements of genuine recessive albinos. All my control series confirmed this classification. It therefore awakened my curiosity when I found that a relatively large number of individuals in certain well-defined experimental series were pigmented.

The modification in question was first discovered in a number of mice of the first generation which were born in the hot and humid environment. At first I paid no attention to the appearance of a creamy coat color in these mice, thinking that it was simply due to lack of cleanliness. This explanation proved to be incorrect. It was impossible to remove the color with any solvent. The possibility that particles of rust from the wire net were to blame was refuted by the iron test. The color commenced at the roots of the hairs. Doctor Hagedoorn, who is an expert on coat colors of mice, was kind enough to examine my mice and expressed as his opinion that they were truly pigmented.

The pigmentation made its first appearance when the mice were about 6 or 7 weeks old. In the hot room only mice belonging to the first "descendant" generation acquired pigment. It was later found that also a few mice which at ordinary room temperature had been exposed to strong electric light had become pigmented. All the pigmented mice were males. I was further able to ascertain that 75 per cent of them were individuals whose body weight was above normal. In the majority of cases the pigmentation was confined to the rump, ending in front along a rather sharp angular line. In a few instances a median, longitudinal creamy streak was observed on the belly. The region round the external sexual organs was deeply yellow. In other parts the pigmentation had a creamy tinge. It persisted mostly throughout the observed period of life of the animals. As might be expected, my attempts to test whether the pigmentation would breed true were negative.

For the microscopical examination of the hairs I employed Werneke's (14) method. The hairs were covered on a slide with a drop of glycerol and carefully heated in order to drive out air enclosed in them. I found that, primarily in the woolly hairs, golden yellow pigment granules were present in small numbers in the medullary part of the hair in places that corresponded to the position of pigment granules in yellow

mice. The grouping and dimensions of the individual granules were also identical. These groups of pigment granules extended as a rule along the whole length of the hair. In some hairs from the colored part of the coat these histological characteristics were missed, showing that these hairs were albinotic. The hairs from control mice were empty as far as genuine pigment granules are concerned. Occasionally, however, I could observe, near the base of a white hair, formations that answered the description Onslow has given about the "ground substance" in albino hairs (15).

The fact that the food of my mouse colony to a large extent consisted of yellow corn meal, which as known is rich in carotinoids, necessitated a few experiments directed to test the possibility that the yellow color was of such an origin. Its insolubility in ether and petrol ether seems to prove that neither carotinoids nor any lipochrome substance could be responsible for the yellow coloration. The albino rat has further been found to be devoid of carotinoids (16) and by analogy we may conclude that albino mice behave similarly in this respect.

A quantity of pigmented hairs was boiled with a 0.2 per cent sodium hydroxide solution for a while. After decanting the solution the process was repeated with a fresh amount of diluted lye. The two filtrates were poured together. The resulting liquid was bright yellow with a greenish tinge. After adding to one part hydrochloric acid and to the other saturated ammonium sulphate solution a greyish dark precipitate was thrown down in both cases. I am doubtful whether this could have been the "melanin." The amount was too small for chemical examination. Gortner (17) states that he has been able to obtain substances which answer the usual description of melanins, i.e., solubility in alkalies and insolubility in acids and neutral solvents from various keratin materials also from recessive albinos. He thinks that these substances do not belong to the true melanins. Gortner says further that he believes that most proteins contain a nucleus which, under proper conditions, may give rise to pigment.

I wasted much time in futile attempts to demonstrate the presence of tyrosinase in skins from my pigmented mice and also from a number of mice of varying age—a few days to several weeks—which were reared from birth in the "tropical" room. I adopted for this purpose Onslow's technic (12) in preparing the skin extracts. All my tyrosinase tests were negative. The presence of oxidases reacting with polyphenols could be demonstrated in the "heat" and "light" mice but the

intensity of the reaction in these series did not differ from that of the control mice.

Notwithstanding my negative results in demonstrating tyrosinase in the skins of the pigmented mice I am inclined to the view that enzymes —tyrosinase or others—which by reacting on protein substances in vivo may produce pigmentation were not entirely absent in either series of my material. I believe that the enzyme was present in all the animals in such a diluted state that it was destroyed during the process of extraction. If we assume that the optimum temperature for pigment production is relatively high, we may understand that the attenuated enzyme may be able, at higher temperatures, to react with the protein substrate while at lower temperatures it fails to do so. In this discussion we are primarily concerned with skin temperatures. As the height of skin temperature may be considered to be a joint product of internal and external factors we may comprehend the limitation of the colored mouse modification only to certain series of animals. It is possible that some correlation may exist between the pigmentation of the mice and factors that control the internal metabolism of the same. An incidental observation regarding the difference in reaction toward acetonitrile found in a few pigmented individuals which will be reported in the next paper seems to indicate that this may be the case. The fact that lecithin acts as an inhibitor on tyrosinase (18) may further have some bearing on the problem, since I have observed in some work in actual Tropics—as yet unpublished—that the lecithin of the blood undergoes a decided drop in hot climate.

I have recently found opportunity to observe in actual tropical climate a colony of white, pink-eyed rats, the ancestors of which several years ago were imported from Europe. These ancestors are said to have been pure white. At the present time the majority of the rats exhibit a fur of light yellow coloration, which is widely distributed over the coat. Among these pigmented rats the female sex is also represented but in a less degree. By microscopical examination the hairs from the pigmented rats show golden yellow granules identical to the ones I found in mice. Rats which since birth have been exposed to circulating air and whose cooling power was enhanced showed the pigmentation in a markedly less degree.

Reviewing our results on hair growth and pigmentation of mice we are justified in assuming that these closely allied processes are dependent on chemical reactions with different temperature coefficients.

SUMMARY

1. Mice which are suddenly transferred from a hot to a cool environment respond quickly to this change by a stimulated hair growth. No change of hair growth was found in mice that for 2 months had been exposed to humid heat. It is suggested that failure to observe the relative moulting of the mice may have obscured possible changes in direction of a thinning of the coat. Supplementary evidence is presented from observations on man to prove that the hair growth is more rapid in a cool environment.

2. The hair of recessive albino mice may, when exposed to humid heat or to the radiation of strong light acquire the power of producing pigment. This is in the beginning limited to individuals of a certain type, but as supplementary observations on rats indicate, may in subsequent generations extend to other individuals.

3. It is suggested that the prevalent theory that recessive albinos lack the pigment-producing enzymes is false and that failure to extract this enzyme may be due to its presence only in minute quantities.

4. It is further suggested that the chemical processes that control the growth and the pigmentation of hair may possess different temperature coefficients. While cool climate seems more congenial to hair growth the optimal temperature for pigment formation appears to fall within the range of tropical heat.

5. Finally, it is suggested that the small amounts of color-producing enzyme that are supposed to be present in the skin of albinos may become active only in a climatic environment in which the skin temperature of these animals approaches the optimal temperature for the reaction of the enzyme. The possible coöperation of metabolic factors is mentioned.

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STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

IV. Effect of Light and Heat on the Resistance of Mice to Acetonitrile

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In the first one of this sequence of papers (1) evidence has been advanced to show that the growth of mice undergoes definite changes in a new climatic environment. Exposure to strong light at ordinary room temperature stimulated the physical development of mice, while on the other hand confinement in humid heat acted in the opposite direction. In the second paper (2) an attempt was made to attribute this retardation of growth solely to the requirement, in the interest of the energy balance of the animals, of an enlarged cooling surface. The possibility that growth might have only indirectly depended upon heat regulation by way of the effect this latter may have exerted on the activity of endocrine organs, primarily of the thyroids, was not considered. Since a retardation of general growth may result, as well known, from hyperthyroidism the possibility cannot *a priori* be excluded that the slow growth in the hot environment might have been caused by the presence in the blood of excessive amounts of the thyroid hormone. In this paper we will try, as far as the small material at our disposal will permit, to show that this was not the case. During the course of my investigations a considerable number of experiments was performed on the resistance of mice to acetonitrile, which has been found to be a convenient method of testing the thyroid activity. Most of these experiments have, however, been discarded, because the rule in testing the resistance to above-mentioned poison of injecting on the same day the same dilution of acetonitrile in mice of the main and the control series was not strictly adhered to in these experiments. It has been recently demonstrated (3) that the chemical undergoes changes in effectiveness with time even in unopened bottles. Only those experi-

ments in which these precautions were observed will, therefore, be reported.

In determining the thyroid activity of animals several methods are available. *a*, Comparing the relative increase in weight of the glands. Robertson (4) found that coincident with the principal cycles of growth of mice the thyroids increased in weight at a faster rate. This increase was more rapid in male mice which also exhibit a greater velocity of growth than females. It is doubtful, considering the small size of the thyroids in mice, whether it would have been possible to observe small deviations in the growth of these glands, such as possibly occurred in my environmental series. *b*, Estimating the iodine content of the thyroids. According to extensive observations published by Seidell and Fenger (5) the iodine content varies inversely with the size of the glands. Of great interest for our present inquiry is further their statement that both—size and iodine content—follow seasonal curves which for weight reaches a maximum in spring, synchronous with the minimum of the iodine curve. No figures are given on the body weight of these animals—sheep, cattle and hogs—but we may infer that the greatest increase of weight takes place in summer. *c*, Comparing the histological appearance of the thyroids. It has been found that the increase of iodine content of the glands coincides with typical changes of the vesicle epithelium and in the amount of colloid. The former becomes cuboidal and the vesicles themselves are distended by the colloidal content. Such changes, generally accepted as indicating a resting condition of the thyroid, have been observed by Mills (6) and by Stoland and Kinney (7) to result from exposure of animals to high temperatures. The latter investigators further observed that the toxicity of thyroid doses was enhanced by heat. *d*, Determining the resistance to acetonitrile. The three methods referred to above are undoubtedly useful in determining the condition of the thyroid gland itself. It is problematical, however, whether they are suitable for investigations which are primarily concerned with the relative amounts of thyroid hormone which are circulating in the blood. The acetonitrile method offers in this respect decided advantages. As known, it was originally introduced by Hunt as a measure of the extent of oxidative processes in the body (8). The rapid breakdown of the $CH_3 \cdot CN$ molecule with freeing of large amounts of the toxic hydrocyanic acid, resulting in diminished resistance, which was found in mice that had received repeated doses of ethyl alcohol, was explained by Hunt as commensurable with the acquired power of oxidizing the ethyl and methyl groups

at a faster rate than normal. The fact that the low oxidation rate, supposed to exist in inanition, was accompanied by an increase of resistance to acetonitrile seemed to support this view (9). In the hands of its originator, the acetonitrile reaction has later, as well known, been developed to a measure of the thyroid activity, in the first place of exogenous origin, but also of the normal physiological activity of the thyroid in the body (10). It seems to be fully established that the changes in oxidative power of the system Hunt discovered by his method are indirect effects of these physiological states on the thyroid. Since the chapters are not yet closed regarding the nature of the effect of the thyroid hormone we are not as yet in position to understand the mechanism of the acetonitrile reaction. It is believed by Hunt that some kind of neutralization process takes place between the two. A similar neutralization process presumably occurs normally between the thyroid secretion and various toxic compounds produced by the body metabolism, which act as depressors of oxidation. We know that the thyroid hormone itself stimulates oxidative processes. We possess, however, no information whatever regarding the interaction of these three classes of substances, the thyroid hormone, the toxic metabolites and acetonitrile. That essential differences exist between different species in this respect becomes evident when we recognize the fundamental dissimilarity between the reaction of mice on one side and of rats and guinea pigs on the other side toward acetonitrile after thyroid feeding. While the resistanceof mice is increased after such feeding, a diminution of the lethal dose occurs, as known, in the two other species of animals. Summing up these considerations, we may say that the acetonitrile reaction is an empirical test of thyroid activity and that great care must be exercised in applying results obtained in one species to other classes of animals.

For the understanding of our own results on the effect of climate on the resistance to acetonitrile it is fortunate that Hunt has collected material to determine the seasonal curve of this resistance (11). He found that the fatal doses of acetonitrile were for mice about twice as large in winter as in summer. Hunt doubted, however, whether the environment in which the mice were reared in winter—a room heated to 75°F.—could be classified as a cool environment. No figures are given for the summer temperature. References to the relative humidity and light conditions are also omitted. Knowing the dry atmosphere prevailing in heated rooms during a cold winter and on the other side the humid heat in the eastern states in summer, we may infer that

considerable differences must have existed between Hunt's summer and winter series so far as the heat output of the mice is concerned. The resistance of rats and guinea pigs was increased in summer, which may be easily understood, when the difference in behavior toward acetonitrile of these animals is considered. Hunt emphasizes the necessity of correlating the resistance with the rate of growth. He cites Südmersen and Glenny (12) who found that guinea pigs grew faster in summer than in winter and that it required a larger dose of diphtheria toxin to kill a guinea pig of same weight in summer than in winter. If Hunt had followed the actual growth of his animals he would possibly have found in mice a retarded summer growth, analogous to the retardation which I found in those of my mice which had been exposed to humid heat. It occurs to me that Hunt's winter series may correspond to my control series and that his summer experiments may in regard to climatic conditions be similar to my heat experiment.

For testing the effect of humid heat on the resistance of mice to acetonitrile I divided a number of litters into four batches, one male and one female group being kept at ordinary room temperature and the two remaining batches, males and females, being transferred to the hot room when 3 weeks old. The food was uniform in both series and consisted, as may be seen from the composition given in the first paper of this series, almost exclusively of food stuffs which according to Hunt's investigations (13) tend to increase the resistance of mice to acetonitrile. When the mice were 3 months old the resistance tests were performed on the same day with the same freshly made dilution of acetonitrile. The injections were given subcutaneously in the same place under the skin of the back. A few days later the same procedure was repeated with two batches of male mice, derived from the same litters, which had been reared at normal room temperature, one in subdued light and the other exposed to the light from two 60 watt "Mazda" lamps at close range. The age of these series of mice at the time of the test was three months. Tables 1 to 3 give particulars as to dosage of acetonitrile, body weight of the mice and result of injection.

The lethal dose in all the series is very small and corresponds to the minimal fatal doses observed by other investigators in this field. How much this low resistance of my mice might depend upon specific qualities of them and how much, as far as the controls are concerned, upon climatic effects is impossible to tell. The fact that the fatal dose reported by European observers (14) is consistently higher than in America may suggest that the latitude may play a rôle in determining the

resistance of mice to acetonitrile. It is possible, however, that simply the degree of toxicity of the acetonitrile preparations used by different investigators is responsible for the variations in fatal dose.

The slight difference we notice in the two first tables toward an increase in resistance in favor on one side of the female mice as compared

TABLE 1

Resistance of male heat mice to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT		RESULT	
	Heat mice	Controls	Heat mice	Controls
mgm.	*grams*	*grams*		
0.10	19.5	22.0	Survived	Survived
0.15	18.5	22.3	Survived	Survived
0.20	24.8	22.9	Dead	Survived
0.22		25.9		Dead
0.25	20.8	25.0	Dead	Dead
0.30	18.5		Dead	
0.35	19.0		Dead	
0.40	22.3		Dead	
0.45	21.7		Dead	

TABLE 2

Resistance of female heat mice to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT		RESULT	
	Heat mice	Controls	Heat mice	Controls
mgm.	*grams*	*grams*		
0.10		21.5		Survived
0.12		22.7		Survived
0.14	15.2	20.3	Survived	Survived
0.16	18.9	17.8	Survived	Survived
0.18	22.8	23.5	Survived	Survived
0.20	16.1	18.7	Survived	Survived
0.22	19.3	19.3	Survived	Survived
0.24	17.4	22.0	Dead	Survived
0.26		19.3		Dead

with the male ones and on the other side of heat animals above controls, is probably too insignificant to deserve any attention. We are, however, justified in concluding that no increase of the resistance to acetonitrile occurs in mice which for the greater part of their lives have lived in an artificially produced tropical climate.

The resistance in the "light" series was decreased by one-third as compared with the corresponding control series. Notwithstanding the small absolute difference, I am led to believe that this result may be accepted as indicating a diminution of resistance in strong light. On account of the small number of animals this conclusion, naturally, requires confirmation. In this connection I take the opportunity of reporting an experiment performed about two months afterwards on seven male mice which all, since they were three weeks old, had been exposed to electric light. Two of these mice belonged to the "pigmented albinos" which have been discussed in a previous paper (15). The animals were about 5 months old at the time of the experiment. The

TABLE 3

Resistance of male "light" mice to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT		RESULT	
	Heat mice	Controls	Heat mice	Controls
mgm.	*grams*	*grams*		
0.09	23.2		Survived	
0.10	22.3	24.2	Dead	Survived
0.11	23.2	20.7	Survived	Survived
0.12	22.0	18.5	Dead	Survived
0.13	20.6	20.9	Dead	Survived
0.14	23.4	21.3	Dead	Survived
0.15	24.2	20.6	Dead	Dead
0.16	24.8	20.6	Dead	Dead
0.17		21.3		Survived
0.18		24.8		Dead
0.19		22.6		Survived

injections of acetonitrile were given a few days after the discontinuance of the light treatment. It is probable that the acetonitrile had at that time lost a part of its strength. This would explain the great difference in fatal doses between these experiments and the previous "light" experiments. One of the pigmented mice was given 0.20 and the other 0.25 mgm. per gram body weight. One of these mice, ear markings omitted in the protocol, succumbed quickly. The other lay in a stupefied condition for 12 hours but finally recovered. The unpigmented mice were not at all affected by doses twice as large. This experiment seems to suggest that metabolic factors, the exact nature of which it would be impossible to conjecture, were coöperating in producing the pigmented modification in my mouse colony.

It would have been highly desirable to obtain exact figures with regard to the effect of heat on the resistance to acetonitrile in later generations of mice which were born in the hot environment. Several protocols of experiments of this kind were discarded for reasons that have been referred to above. I am, however, still in possession of a protocol of an experiment on eight female mice from the third generation which were born in the hot room. These tests were performed about the same time as the tests on the pigmented "light" mice, which have led us to conclude that the stock solution of acetonitrile had lost part of its effectiveness. The age of these "descendants" of the third generation was 3 months, the same as that of animals which were subjected to the test after having lived in the hot environment from 3 weeks of age. Their body weights are very much lower. In table 4

TABLE 4

Resistance of female heat mice in the third generation to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT	RESULT
mgm.	*grams*	
0.10	17.0	Dead
0.15	14.9	Survived
0.15	16.0	Survived
0.20	15.0	Survived
0.25	12.4	Dead
0.30	16.4	Survived
0.33	15.0	Dead
0.35	18.2	Dead

the data are collected for this incomplete experiment. The variability of the lethal dose is conspicuously high. The data, even without any correction for the change in toxicity of the chemical, do not lend any support to the supposition of a diminution of the resistance to acetonitrile in a hot climate. It rather appears as if, actually, the effect were an opposite one, which would tend to accentuate the slight indications in a similar direction which we received in our better controlled heat experiment of this sort.

Translating our results into terms of thyroid activity according to our accepted principles we may say that confinement of mice even for several generations does not increase this activity but rather acts in an opposite direction. Exposure to strong light seems to cause a slight diminution of the thyroid hormone in the blood. These results seem

further to be in full accord on one side with the seasonal curve of thyroid activity that Hunt has reported, and on the other side, with regard to the heat experiments, with the findings of investigators who have furnished histological evidence of a resting condition of the thyroid in a hot environment. Whether these changes form a link in the adaptation mechanism of the body to a tropical environment is impossible at present to say. It is possible that the demand for active thyroid hormone is diminished in the Tropics, where the "inside fires are banked." Observations that an increase in external temperature enhances the toxicity of thyroid medication seems to indicate that the body might in a hot climate instinctively lessen the flow of thyroid secretion into the blood. Although it must once more be emphasized that experimental results obtained on animal species cannot directly be applied to human medicine, we are nevertheless compelled to be sceptical about certain "clinical" reports of tropical practitioners who announce wonderful benefits from the use of thyroid preparations on various conditions of debility.

The question which was the immediate origin of this ingress into the field of internal secretion, namely, whether the retardation of growth of mice, which are adapted to a humid and hot environment, may be attributable to a stimulation of thyroid secretion must be answered in the negative.

SUMMARY

1. The resistance of mice to acetonitrile is not augmented in humid heat; the small material at hand indicates on the contrary a slight diminution of this resistance. It is, therefore, suggested, in the light of accepted principles regarding the correlation of acetonitrile resistance and thyroid activity, that the tropical climate—at least for mice—may lessen the demands for thyroid hormone in the body.

2. The retardation of growth of mice which has been observed to occur in humid heat is not attributable to a stimulation of the thyroid activity.

3. The resistance of mice exposed to strong light at ordinary room temperature is slightly diminished.

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STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

V. Effect of Humid Heat on the Blood Morphology of Mice

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Probably the first contribution of scientific value to tropical physiology was made in the realm of blood morphology. Among medical as well as laymen the conception had been prevalent that the tropical climate as such may affect the blood conditions and cause a "tropical anemia." A number of short, more or less well-controlled investigations was therefore made on the blood picture of individuals who for a time had resided in the Tropics. They all seemed to refute the existence of an anemia of purely climatic etiology. These results have at a later date been confirmed by more extensive and systematic investigations (1). As to the red blood corpuscles, some observers have gone so far as to postulate that, in analogy with experiences in high altitudes, the number of erythrocytes may as a rule be increased in healthy inhabitants of the Tropics (2). Still less consensus of opinion exists with regard to the white blood corpuscles. While most reports (3) indicate that their number generally lies in the lower part of the range for temperate climates, other investigators (4) are inclined to the view that a slight hyperleucocytosis is produced by heat. A few observations (5) which point to the existence of lymphocytosis and a shift to the left of the Arneth index, have been criticised as paying not sufficient attention to the possible effects of parasitological ailments, and on the other hand not considering various climatic conditions and seasonal variations. It is surprising that, so far as I am aware, no attempts have been made to approach these problems by experiments on animals, living for longer periods of time in an artificially produced tropical environment. Of considerable interest in this connection is, however, a research series published by Murphy and Sturm (6). These investigators found that after exposure for 5 minutes to dry heat—55 to 65°C.—the blood picture of rats, mice and guinea pigs

underwent decided alterations. The white blood count dropped and this change affected equally the mono- and poly-nuclear cells. During the days succeeding the transfer back to a cool environment the curves commenced to rise and reached finally levels far above the original counts. The lymphocytes were primarily affected by this compensatory change. It was further found by Nakahara (7) that after the heat treatment numerous degenerated cells were present in the spleen and in the lymphatic glands. An enhanced cell proliferation was discernible during the after-period.

It occurred to me that my healthy and well-controlled mice series might offer an ideal material for studies on the effect of humid heat on the blood morphology. Insofar as time and available animals permitted I therefore gave my attention to this line of research. As the customary method of drawing the blood from the tail vein of mice seemed to me to be open to criticism, I decided to let my blood samples represent as large part of the total blood volume as possible. I therefore killed the animals by cutting their throats and collected the freely flowing blood on a watch glass. After quick mixing of the blood the blood pipettes were filled within a few seconds after the operation. A few times control countings were made by filling two pipettes from the same blood sample with satisfactory agreement between the results. While thus the method, with regard to blood counts, was fully satisfactory and obviously superior to the employment of peripheral blood for the purpose, I regret to say that the blood smears—four or five from each mouse—did not fulfil my expectations. Differential counts obtained from different slides for the same animal did not agree. Most probably the time consumed in preparing the slides was sufficient for the blood cells to commence to settle down. Stirring did not completely restore the original distribution of different classes of white cells. Under those circumstances it would have been waste of time to continue differential counts, the results of which would have been uncertain. I gained, however, the impression that the mononuclears —especially the large ones—were relatively numerous in both series, heat and control. In the latter they seemed to exceed the figures published by other observers (8). I abstained also from a classification of the polynuclears, since this apparently offers considerable difficulties in mice. I received from a few comparative observations the impression that more rod nucleated polymorphonuclears in proportion to polymorphonuclears with a vacuolized or pycnotic nucleus were found in the mice from the hot environment than in the controls.

Red blood corpuscles. In ten control mice the average red count was 10.14 million with a standard deviation of 0.75 million. Since the red counts of males and females agreed in all respects I did not attempt to treat them separately. The sexes were furthermore equally represented in all series. The number of erythrocytes of the controls agrees well with standard figures reported by Klieneberger and Carl (8), average 9.73 million, and by Lange (9), males 9.48 and females 9.18 million. In 16 mice which had been exposed to heat since they were 3 weeks old the red count was 11.16 million with a standard deviation of 1.16 million. An increase of 10 per cent had consequently taken place in the average red count of the heat mice. The chance that this increase was accidental is one in 142. If the eight red counts that were performed on various other series of "immigrant" heat mice are added the average of the 24 variates remains the same, 11.13 millions. The average of eight red counts on mice which were born in the hot environment was 12.10 million which seems to indicate that a progressive change in a positive direction of the number of erythrocytes took place in the "descendant" generations. The greater part of this rise of the erythrocyte curve is doubtless due to inspissation of blood but, considering the synchronous progressive drop in the total white count, which will be referred to below, the possibility cannot be excluded that a real increase of small dimensions might have occurred in the number of red corpuscles in those mice which were adapted to a hot and humid environment.

White blood corpuscles. The table below gives a comprehensive idea of the changes of the total white count in mice which were reared in the "tropical" environment. The average count for the control animals closely resembles the standard figure given by Klieneberger and Carl (8) 7400. The number of white blood cells of "immigrants" in the hot room is lower but the probable error of the difference is too large to allow any definite conclusion to be drawn. Both series of the first generation born in the hot environment—one kept in subdued light and the other exposed to the light from a 100 watt "Mazda" at close range—agree closely with regard to their avarage white count. The chance that the difference between the controls and these "descendants" would be accidental is one in 100. The white count of the fourth generation is still lower.

A correction for the inspissation of the blood in the mice from the "tropical" room would only serve to accentuate the difference in white count between the controls and the heat mice. We seem to be justified,

therefore, in assuming that the heat tends to lower the number of white blood corpuscles, which is in agreement with the majority of observations on human blood in the Tropics. We are led by these results to believe that the organs concerned in the formation of white blood cells are sensitive to tropical heat. In the experiments from the Rockefeller Institute which we have referred to above, the extreme heat stagnation lasted only for a very short time. Under the climatic conditions that prevail in the Tropics and which we have imitated in these studies the moderate heat stagnation is continuous. The results seem to be the same in both cases with regard to the effect of the heat on the cellular activities that control the supply of white blood cells. It is regrettable that I omitted to consider the effect on the white count a transfer of the mice to a cool environment would have caused. It is

TABLE 1

Average white count with standard deviation in control and four series of heat mice

SERIES	NUMBER OF VARIATES	MEAN	STANDARD DEVIATION
Control mice	38	7300	2170
Heat mice, "immigrants"	13	6880	3170
Heat mice, "descendants"			
1. generation subdued light	25	5730	2010
Heat mice, "descendants"			
1. generation strong light	25	5550	2050
Heat mice, "descendants"			
4. generation	25	4950	1730

possible that those investigations of human blood in the Tropics which have showed a hyperleucocytosis—supposing pathological factors were absent—have been made primarily during cooler respites and that the increase of white cells they indicate is analogous to the hyperleucocytosis which Murphy and collaborators observed as an after reaction to the hot air treatment of their animals.

SUMMARY

1. Progressive changes in direction of an increase of the number of erythrocytes were observed in mice which for the greatest part of their life or since birth had been confined in humid heat. It is suggested that this change is primarily due to inspissation of blood.

2. Progressive diminutions of the white count occurred in a number of generations of mice which were reared in humid heat. It is suggested

that this change was produced by high sensitivity to an increase of temperature exhibited by organs concerned in new formation of white blood cells.

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STUDIES IN PLACENTAL PERMEABILITY

II. Localization of Certain Physiological Activities in the Chorionic Ectoderm in the Cat

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The first communication of this series concerned a group of experiments the results of which were of interest in two of the many phases involved in the complex question of nutrition of the fetus. In these experiments it was found that both iron ammonium citrate and sodium ferrocyanide, when given in a solution containing equal amounts of each salt, passed through the easily permeable maternal endothelium in ten minutes. Both of these salts penetrated the syncytial layer of the chorionic ectoderm in about two hours, and finally the ferrocyanide reached the fetal circulation in four or five hours while the citrate had not done so at the end of ten.

When the placentae and membranes from these animals were fixed in an acid medium Prussian blue granules were precipitated in characteristic regions of the chorionic ectodermal layer. Each giant cell was surrounded by a ring of blue granules and both the portion of the syncytium bordering the maternal endothelium and some of the fine strands of cytoplasm which appear between the large vacuoles, seen in the fixed material, also contained the Prussian blue. The distribution of the granules of Prussian blue indicated the distance to which the citrate had penetrated. The area in which these granules were found seemed therefore to have special significance, and this finding suggested that perhaps this was an area in which some particular physiological activity took place, perhaps the breaking down and resynthesizing of substances finally intended for fetal consumption. On the other hand the fact that these two salts, which diffuse at approximately the same rates through inert membranes, should show such different properties when brought into contact with the placental membrane, suggested that there must be some factors involved in placental inter-

change other than the simple diffusion of molecules in the physical sense. Several suggestions were made as to the possible nature of these additional factors, the most plausible being that the chorionic ectoderm was capable of breaking down the citrate in such a way that the ferric iron no longer reacted as such.

Two very interesting papers, appearing too late to be referred to in the first number of this series, are important in relation to the two principal conclusions which have been outlined above. Wislocki (37) has studied the distribution of trypan blue in the placenta of the cat, after administration to the mother in repeated doses. It may be seen by looking at his figures 14 and 15, plate 4, that the distribution of the droplets of trypan blue in the syncytium of the fetal ectoderm is quite similar to that which I have described for the Prussian blue. This fact is interesting because it demonstrates that this particular area of the fetal chorionic syncytium is capable of reacting to at least two types of substances, one of which is an inert colloid and the other a diffusible salt. From these observations it may be safely suggested that much of the normal physiological activity of the placenta is localized in this area.

The other paper which is of such particular interest in this connection is one by Edelstein and Ylppö (7). These authors made comparative studies of the blood of the mother and the newborn child. They determined the amount of sodium and potassium in both bloods; and found that in all cases the sodium content was greater in the fetal blood than in the maternal. In nine cases they also found that the amount of potassium was larger in the fetal blood than in that of the mother, but there were three interesting exceptions; in two of these the maternal content was much higher, the fetal remaining exactly the same as in the other nine cases. The abnormally high percentage for the maternal blood in these two cases the authors attribute to nutritional factors. In the third case where the usual ratio was not found, the content of the maternal and fetal bloods in potassium was the same. In this instance the mother and child were both syphilitic and the authors suggest that the normal regulating mechanism of the placenta may have been impaired so that the salts could pass more easily, perhaps even as in the case of an inert membrane. They consider that their results give evidence that the passage of salts from mother to fetus is not controlled entirely by the laws of osmosis and diffusion, and suggest that the mechanism of placental transfer is regulated by certain vital cellular activities similar to those obtaining in intestinal and kidney cells.

The interchange of salts and the localization of activities in the placenta are two of the most essential points to be considered in studying the phases of the activities in which that membrane is called upon to mediate. Many possible explanations of these phenomena have been suggested, yet none of these can be entirely correlated with the great mass of evidence which has accumulated on the subject.

This number of the series is intended to examine the reaction of the cat's placenta after more extended exposures to sodium ferrocyanide and iron ammonium citrate than those reported in the first paper, to control the interactions of the two salts by studying them separately, and to further control the use of anesthetics by several new methods. With these facts established as far as possible with the limited material at hand some attempt is made here to determine the exact bearing of these experiments upon the question of placental interchange and to establish their proper relationship to the other work which has been done.

Materials and methods. The animals used were pregnant cats, in the latter half of gestation, the youngest fetus measuring 79 mm. and the oldest 110 mm. The methods employed were in general similar to those recorded in the earlier communication (5), and were briefly as follows: the animals received balanced solutions of sodium ferrocyanide and iron ammonium citrate, the usual strength was 1½ per cent of each salt. The solution was injected into the vein of the fore-leg, the temperature and rate of flow being kept constant. At the termination of the experiment the animal was killed, the uterus opened and the fluid carefully withdrawn from the amniotic sac with a syringe and needle. The fluid was then tested for the experimental salts. The fetuses were removed and their abdominal cavities opened. If the bladder was distended, it was handled as in the case of the amniotic sac. The placenta and membranes were fixed in Bouin's fluid, the acetic acid in the mixture precipitating the Prussian blue. In the present series of experiments some placental tissue was also fixed in neutral formalin and 95 per cent alcohol. In the analysis of the amniotic liquid and fetal urine, solutions of ferric chloride and ferric sulphate were used to test for the ferrrocyanide, and ferrocyanide was used to determine the ferric iron. The longest experiment in the first series was 10 hours. In this series the duration of the experiments was extended to 14, 18 and 24 hours. No animals were kept exposed to either or both of the two experimental salts longer than 24 hours. These experiments of 14, 18 and 24 hours were on animals which had been nephrectomized under

ether anesthesia, the salts injected intravenously and the animals allowed to recover. They remained entirely normal in appearance up to about 20 hours when they became slightly drowsy.

A few other experiments using each of the two salts alone were carried out to determine whether the sodium ferrocyanide had any part in preventing the passage of the iron ammonium citrate through the placenta. Finally several experiments of 4 to 10 hours duration both on animals which had been nephrectomized and on others with functioning kidneys, were done under urethane. These experiments were carried out as controls for those done under ether, since urethane has been found to be practically without effect on respiration, circulation, etc.

Experimental results. On histological examination, the placentae from those experiments in which a combined injection of sodium ferrocyanide and iron ammonium citrate was given, presented the same general features as those described for the shorter experiments. In all of them in which the fetuses were alive when the animal was sacrificed the Prussian blue could be seen surrounding the giant cells in a definite blue ring, a few granules of blue could occasionally be seen within these cells, but these were quite rare.

The maternal endothelial cells contained a few blue granules within their cytoplasm, these were however less in number than those found in experiments of 2 to 4 hours duration. The striking finding was again the appearance of the syncytium of the chorionic ectoderm: here the Prussian blue was precipitated in clumps and fine lines within the cytoplasm adjacent to the maternal endothelium and extended between the nuclei, sometimes as far as the end of the nucleus nearer the fetal vessels. The border of the chorionic ectoderm adjacent to the fetal vessels always remained free of the Prussian blue.

In most of the experiments having a duration of more than 8 hours there was evidence of some precipitation of Prussian blue in the living placenta, but this was never extensive. In these cases some of the tissue was always fixed in neutral formalin or alcohol and care was taken that no acid reagent came in contact with the sections during staining. In this way the amount and distribution of this precipitation has been determined. Sections from these placentae showed the usual distribution of Prussian blue but the amount was very greatly reduced, usually there were a few fine granules between the maternal endothelium and the chorionic ectoderm and others distributed in the border of the syncytial layer of the chorionic ectoderm adjacent to the maternal circulation. These observations are interesting because they indicate the

involvement of some factor that brings about a partial precipitation of the Prussian blue during the life of the cells. That this is true, (i.e., that the chorionic ectodermal cells are still living when the precipitation takes place) is evidenced by the totally unstained state of the nucleus. In all histological preparations from the experiments of this series and those reported before, a few nuclei can be found that have stained a diffuse blue; these unquestionably indicate cellular death, but there has been no evidence of any increase in the number of stained nuclei in the experiments of longer duration or in those in which the maximum degree of intra-vitam precipitation has been found. This observation indicates that the degeneration of the protoplasm and the consequent production of acid can not be admitted as a likely explanation of the partial precipitation of the blue granules.

Turning now to the experiments of longer duration in which the citrate was used alone, only three animals were available for this purpose. In two of them double the usual dose of the iron ammonium citrate was given and the animals sacrificed at 12 and 18 hours respectively. In neither case was there any evidence of ferric iron in the urine of the fetuses or in the amniotic fluid. In the third it was thought interesting to try a very large dose with the hope of overloading the placental mechanism and then finding the passage of ferric iron to demonstrate that the membrane as such was permeable to the simple salt. In this animal $4\frac{1}{2}$ grams were injected intravenously into the circulation of the mother in a 2 per cent solution. The animal was somewhat toxic as the result of this large dose and after about 5 hours became quite sleepy. Sacrificed at 12 hours the fetal urine in one bladder showed what was thought to be a trace of ferric iron but the test was questionable. The amniotic fluid was frankly negative. However it seems probable that a very small amount of ferric iron had passed to the fetal circulation and had been excreted as such by the fetal kidneys.

The results obtained when urethane was used as the anesthetic were entirely comparable to those found in experiments performed on animals under ether anesthesia, or on decerebrate animals, as described in the first paper of this series (5).

Discussion. It seems evident from the experiments reported above that the precipitation of the Prussian blue granules indicates the course of the sodium ferrocyanide and the iron ammonium citrate through the placental tissues, and that the latter salt under consideration is in some way arrested or changed during this passage so that it does not enter

the fetal circulation in such a form that it can be detected by the reactions used. Further it seems certain that there is some precipitation of the Prussian blue in the living tissue, because such an intra-vitam formation of the characteristic blue granules was not accompanied by any findings indicative of a moribund condition of the cells. If one thinks of this reaction as resulting from the intact molecules of the two salts by the intermediation of an acid, the question arises as to the possibility of acid formation in the living cell; and these cells are unquestionably still alive even though they may be injured to a considerable extent. Whether enough acid can be produced in protoplasm as the result of injury to cause such a precipitation without killing the cell is most difficult to answer. It hardly seems possible in the present case because distribution of the Prussian blue granules was so general throughout the placenta that had the cells been dead the entire placenta must have ceased functioning. It was interesting to find that the death of the fetus did not seem to increase the precipitation of the Prussian blue. On the whole the explanation which seems most logical is that the chorionic ectoderm has the power of altering the chemical structure of this compound, and that one stage in this process results in the change of the iron ammonium citrate in such a way that a compound is formed which would combine with the sodium ferrocyanide and form Prussian blue. Harvey and Bensley (10) have suggested that ammonia might be removed from iron ammonium citrate in the blood stream and the resulting ferric citrate be free to combine with the cyanide; perhaps the reaction taking place in the placenta is similar to this. If this hypothesis were true then longer exposure of animals that had received a single dose given at the beginning of the experiment should show increasing amounts of the Prussian blue formed during life. This has been found to be the case. Again it must be asked what prevents the remaining citrate which is evidently free in the blood stream and unchanged in the ectoderm, from passing to the fetal blood stream. It seems necessary to postulate two forces, one which stops the citrate in its passage through the placenta, and another that is capable of changing it in such a way that a ferric citrate is formed. It is also possible that still further or perhaps different reactions also take place within the cytoplasm of the chorionic ectoderm.

There is also the possibility that the Prussian blue has been formed in the blood stream and the granules actively phagocytized by the tissues of the placenta. The molecules of Prussian blue are as small as those of trypan blue, but it is well known that those of the former tend

to form aggregates which are larger than particles of india ink. It is evident therefore that if flocculation has taken place the Prussian blue would not be phagocytized while if the chorionic ectoderm had access to the freshly precipitated molecules a storage similar to that of trypan blue might result. That this is not the case is indicated by the rapidity with which the Prussian blue reaction takes place (8 to 10 hours), while it requires 2 to 3 days for the storage of trypan blue to take place; by the lack of storing of Prussian blue in the numerous cells elsewhere in the body which do store vital dyes; and finally by the absence of Prussian blue granules in the giant cells which are the most active storehouses of true vital dyes. Whatever may be the explanation of this reaction between the two salts and the formation of Prussian blue within the living cytoplasm of the chorionic ectoderm, it seems certain that it cannot be the cause of the impermeability of that structure to the one salt while the other was able to pass to the fetal circulation. This is further evidenced by the fact that the ferrocyanide could be detected in those animals in which there was the greatest precipitation as well as in those in which there was much less, and there was invariably a marked increase in the amount of Prussian blue found in the chorionic ectoderm after acid fixation. That this reaction is of very great importance can not be doubted, but further investigation will be required to determine its meaning and relation to the activities of the placenta.

There is sufficient evidence to conclude that the change which the iron ammonium citrate evidently undergoes is effected in a definite and given area of the tissues intervening between the maternal and fetal circulations. This localization is of considerable interest because one of the most discussed problems in fetal nutrition is whether the foodstuffs used by the fetus are actually prepared in any part by the placenta, or whether they are derived by diffusion directly from the maternal blood plasma, having been prepared by the maternal tissues. A very large group of workers has supported the idea that the placenta was actively engaged in the metabolism of the fetal foodstuffs, and a few have in addition specifically localized these activities within the placenta itself.

Claude Bernard (3) showed that the maternal part of the rabbit's placenta contained large amounts of glycogen. This was substantiated by Driesen (6) in the human. The fetal part of the placenta has also been found to give a glycogen reaction by Langhans (18), (19), and by Zuntz (38).

Ascoli (1) found in a large number of serological experiments on foreign proteins that these could not pass the placenta as such, he also found that the placenta contained proteolytic ferments. From these and other experiments he concluded that the placenta was capable of breaking down proteins, but was unable to decide whether these were attacked in the form of total protein molecules or as some intermediate product.

Hofbauer (12), (14) studied the distribution of fat in the human placenta and found that the part of the syncytium adjacent to the maternal circulation contained no fat droplets but they were present in the basal part of the layer. He compares this finding with the distribution of fat in the intestinal cells during absorption of food and concludes that the two processes are strikingly similar. Further Hofbauer fed animals and pregnant women fat stained with Sudan III and he claims to have found that both maternal and fetal bloods contained the dye but the droplets in the placenta and in the fetal tissues were always unstained. On the basis of his histological findings in the human and these experimental findings, he concludes that fats are broken down in the outer layer of the syncytium and resynthesized to form a fat corresponding to that of the fetus. Hofbauer (16) obtained very similar results in studying the absorption of iron, finding that the iron granules detected by microchemical reactions became more and more evident as the fetal vessels were approached. He also considers (15) that the presence of ferments in the placenta has assisted in substantiating his views as outlined above. Kehrer (17) and Heyman (11) also believe that the processes in the placenta are analogous to those in the mechanism of food absorption of the intestine.

The extent to which ferments are involved in placental interchange is still uncertain. Bergell and Falk (2) conclude that there are sufficient enzymes in placental tissues to account for the albumin requirements of the fetus. These enzymes they consider as unquestionably intra-cellular. And they conclude that the placenta is a metabolistic organ, regulating the fetal nourishment, and that it is more analogous in function to the liver than to the intestine. Frank (8) on the contrary after a careful study of placental and fetal ferments and enzymes comes to the conclusion that his results afford no support to the view that the placenta acts as an organ of digestion for the fetus.

During the last four or five years a large number of communications has brought important additional information to assist in analyzing the question of placental transfer. These papers have dealt with the

chemical analysis of the bloods of mother and fetus; modern methods having been developed which permitted the determination of a variety of metabolic substances and excretory products in very small amounts of blood. Slemons and Bogert (29) have established that the maternal and fetal bloods have approximately the same content in uric acid, Slemons and Morriss (30) have obtained similar results in comparative studies of the urea and non-protein nitrogen, in the two bloods, and Plass (25), (26) found an equal content in creatine and creatinin. These observers conclude that the exactly similar content of the fetal and maternal bloods indicates that the excretory products pass the placenta by diffusion. Morriss (21) found that the blood sugar was slightly higher in the maternal than in the fetal bloods; this he interprets as meaning a rapid storage or metabolism of sugar by the fetus and does not think it indicates any regulatory mechanism in the placenta. Morse (22) has found that the amino-acid nitrogen is higher in the fetal than in the maternal blood serum. To explain this he assumes a capability of the placenta to "absorb" the amino-acids, a mechanism similar to that suggested by Van Slyke (33), (34), (35) in the case of the removal of amino-acids from the blood stream by the tissues. From this evidence which indicates a one-sided permeability he is forced to postulate a mechanism in the placenta for retaining the amino-acids in the fetal circulation.

Slemons and Stander (31) found that the maternal blood contained a much larger amount of lipoids and fats than the fetal, and Mendel and Daniels (20) found that stained fats would not pass from mother to fetus in the case of pregnant rats. Slemons (27), (28), from these two groups of experiments, concludes that the fats and allied substances do not pass the placenta, and that the fetus constructs its own fats from the carbohydrates supplied it by the mother. The view that the fat molecule as such does not pass the placenta has been generally accepted. Oshima (24) in particular has confirmed this by ultramicroscopic studies on the fat content of maternal and fetal bloods. But that some allied substances can traverse the placenta is indicated by the work of Hofbauer (13) and of Thiemich (32). They fed cocoa-fat to pregnant animals and found fatty acids characteristic of this fat in the fetus. Finally with reference to the transfer of iron practically all the evidence still rests on the microchemical observations. The present status of the question is well stated by Slemons (27) as follows:

> With regard to iron it is impossible, at present, to affirm what arrangements are made for its transportation through the placenta. This intricate and unsolved

problem occupies a unique position among the factors of fetal nutrition. Stored in the newly born infant there is a large quantity of iron, so large, indeed, that the quantity is proportionately much greater than in the adult. The purpose of this storage in the newborn, Bunge believes, is to compensate for the inadequate amount of iron in human milk.

These various observations have, I think, gone far toward establishing the importance of diffusion in the passage of certain substances particularly the excretory substances of the fetus; but on the other hand the results regarding sugar, amino-acids, fats and iron tend to suggest a regulatory mechanism in the placenta itself. As we have seen, the work of Edelstein and Ylppö (7) indicates a regulatory mechanism for salts, while Cohnstein and Zuntz (4) come to the opposite conclusion.

Nicloux (23) found that alcohol and ether were governed in their transmission through the placenta by the laws of osmosis and diffusion, and from these observations he decided that all diffusible substances including true solutions of soluble salts would be governed by the same laws. In that part of his conclusion which is based upon his own observations there is no possibility of adverse criticism, but in the extension of his theories to include those salts that had only been reported on by others, the conclusion is made too general.

Kehrer (17) concludes that all serum-salts which are not combined with albumin and protein pass through the placenta easily and obtain entrance to the fetal blood. This transfer he believes is controlled by osmosis and diffusion alone. The question of what he means by the combination of salts with proteins is very interesting because it is conceivable that any and every salt may enter into some kind of combination with some larger molecule of the living system. It is very probable that any salt molecule which could diffuse freely into a cell would leave it equally as readily unless some change had taken place in its physicochemical state after it had gained entrance to the cytoplasm. From this brief review of the conflicting views on salt transfer it is evident that a decision can not yet be made as to whether osmosis and diffusion represent the dominant forces engaged in the transfer of those soluble salts which are normally present in the blood plasma.

The forces of osmosis and diffusion seem inadequate to explain the reaction of the protoplasm of the placental cells and syncytium to many colloids. Goldmann (9) and Wislocki (37) have shown that the placenta is practically impermeable to trypan blue and other acid-azo-dyes, while these dyes are stored within the protoplasm. Why do these molecules, too large to "pass through the placenta," pass through one

side of the lining substance and not through the other? There must be either a difference in the permeability of the two sides of the placental tissue or else the dye must meet with some chemical or physico-chemical reaction within the living cytoplasm which prohibits its further progress.

The conflicting reports which have been briefly reviewed above, and the results so far reported in this series only furnish sufficient basis for a working hypothesis regarding the mechanism of placental interchange. This theory may be expressed in terms of a reclassification which is dependent upon specific reactions of the placenta to specific substances. Three groups may be indicated: *a*, Those substances which are diffusible and which meet with no mechanism in the placenta capable of acting on them; these pass by diffusion from mother to fetus, or in the reverse direction without any mediation on the part of the placenta. This group contains most of the excretory products of the fetus, and large numbers of foreign substances, many of which are highly toxic. *b*, A group of substances to which the maternal or fetal surfaces of the placental barrier are impermeable. Here may be grouped the formed elements which are normally present in the circulation and such foreign substances as insoluble salts (e.g., barium sulphate) and foreign particulate matter such as india ink, cinnabar and bacteria. *c*, Certain substances which meet a definite preformed regulatory mechanism in the placenta. At present this group must include most of those substances which are designed for the fetal metabolism and certain important inorganic salts, especially those containing iron.

This hypothesis emphasizes the idea that the placenta is an apparatus to insure the receipt and retention of sufficient materials for fetal metabolism, and that this is accomplished by the development of certain specific reactions which are probably entirely separate entities in their physico-chemical nature. That the iron ammonium citrate undergoes some change within the chorionic syncytium is indicated by the results of the experiments reported here. But there is very little evidence as to what the nature of the reaction may be save that at some stage a compound is formed that reacts with sodium ferrocyanide to form Prussian blue; with regard to the experiments in which iron ammonium citrate was used alone the evidence does not seem to be sufficient to authorize the conclusion that an excess beyond the normal capacity of the placenta would diffuse through into the fetal circulation, but is only suggestive of that. However even if such an hypothesis be warranted there is still the evident fact that the mechanism is capable of

dealing with an almost unbelievable amount of the salt, so much in fact that experimentally it does not seem possible to examine it because the limit of the amount of the salt that the animal can stand seems to be about equal to that amount which the placenta is capable of handling.

CONCLUSION

The results reported here suggest that the iron ammonium citrate meets a specific regulatory mechanism which is capable of changing quite large amounts of this salt. It seems probable that this mechanism is normally concerned with the control of the passage of iron containing substances, the decomposition of which is necessary for the preparation of iron for fetal use and storage. That this conclusion can only be suggestive and not absolute is clear, but it is certain that the chorionic ectoderm in the cat does react differently to two salts whose diffusion rates are not greatly dissimilar when measured by their reaction to other living membranes.

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STUDIES IN EXPERIMENTAL TRAUMATIC SHOCK

VI. The Liberation of Epinephrin in Traumatic Shock

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There is evidence that during the development of shock there is a general over-activity of the sympathetic division of the autonomic system. Thus, there is acceleration of the heart, dilatation of the pupils, and a generalized vasoconstriction. This latter factor has recently received considerable attention and has been emphasized as an important conserving mechanism. Guthrie (1) has observed that cutting the nerve supply of a limb in a normal animal would result in an increase of blood flow through that limb of 22 per cent, whereas performing the same maneuver on a shocked animal resulted in a rise of blood flow amounting to 76 per cent. Erlanger, Gesell and Gasser (2) found that in shock produced by handling the abdominal viscera there was marked constriction of the limb vessels as the blood pressure dropped, and that not until the pressure had reached 50 mm. Hg. did the vessels of the limb dilate, as determined by their inflow method. Cattell (3) has noted a similar vasoconstriction in the development of traumatic shock. In his experiments, vasodilatation did not occur until, on the average, the blood pressure had been reduced to 65 mm. Hg.

Accompanying this vasoconstriction, and particularly in view of the symptoms of general sympathetic stimulation, it would be not unreasonable to postulate the possibility of an increased activity of the adrenal glands, acting either as an ungovernable and deleterious factor in the development of shock, or as a secondary conserving mechanism, or, conceivably, as an incidental concomitant, without special significance in this connection.

Whether or not such an increased activity of the adrenals occurs has been in dispute. Bedford and Jackson (4) and Bedford (5) published observations tending to show that in shock produced by handling of the intestine, hemorrhage and occlusion of the inferior vena cava, there

is an increased amount of epinephrin in the caval blood at a point opposite the entrance of the lumbo-adrenal veins. Dogs were used, and the method involved opening of the abdomen and a certain amount of preliminary manipulation of the abdominal contents before the supposed control specimens were obtained. The blood was tested for epinephrin by means of rabbit intestinal "strips." The conclusions arrived at by Bedford were: *a*, Increased quantities of epinephrin are thrown into the blood during conditions of low blood pressure and shock. *b*, This is due to hyperactivity—not to depletion of the glands. *c*, The quantity of epinephric material in the blood increases only after a somewhat prolonged continuation of the conditions leading to shock. *d*, The quantity of epinephric material in the blood increases with the prolongation of the period of low blood pressure and shock. *e*, This increased output of epinephrin into the blood may be a last effort on the part of the organism to resist the forces that are tending toward a fatal degree of low blood pressure.

Stewart and Rogoff (6) repeated this work, using essentially similar methods, but were unable to obtain any evidence of increased liberation of epinephrin per unit of time. Shock was produced by intestinal manipulation, hemorrhage, and "peptone" injections, and blood from the "cava pocket" was assayed by means of rabbit intestine and uterus segments. These authors criticise the work of Bedford on the ground that he did not estimate quantitatively the amount of epinephrin per unit of time. As a matter of fact, however, he appears to have taken into account the rate of flow from the lumbo-adrenal veins and, allowing for differences in the rate, there is still evidence in his published figures of increased liberation of epinephrin in the conditions of shock produced in his experiments.

In view of the above conflicting results, and in view of the possible significance of epinephrin over- or under-production as a shock inducing factor, the experiments that I report were undertaken.

Method. Cats were used throughout, and they were anesthetized with ether. Thirteen experiments were performed, of which four were controls, in which the adrenals had been removed prior to the induction of shock. The latter was accomplished by crushing the thigh muscles of one leg. In the majority of cases the sciatic and femoral nerve trunks were cut cephalad to the region of crushing, so as to rule out, as far as possible, the question of reflex nervous stimulation of the adrenal glands. It may be said, in passing, that no striking difference was observed in the two types of experiments and, consequently, any posi-

tive results obtained were not the effect of repeated passage of impulses centrally along the large nerve trunks of the leg.

The heart, isolated from its extrinsic nervous mechanism by cutting the vagi in the neck and removing the stellate ganglia, was employed as an indicator of the liberation of epinephrin into the blood stream. This method has been described by Cannon (7). The stellate ganglia were removed through an opening between the first and second ribs, under artificial respiration. Blood pressure and heart rate were recorded by a cannula in the carotid artery, connected to a mercury manometer. A tracheal cannula was held in place by a tie that included the inferior thyroid veins. It was important to keep the temperature as nearly constant as possible. This was accomplished with sufficient exactness to prevent this factor from having appreciable influence on the results.

Experimental observations. Of nine experiments, excluding the controls, five showed a definite rise of rate as the blood pressure was falling, indicating increased liberation of epinephrin. Three showed either no change or a slight drop in the rate, usually gradual. In one experiment, there was an initial rise in rate immediately after crushing the muscles, due possibly to nervous stimulation, as the leg nerves had not been cut. Following this, the rate dropped to a point slightly below the original rate, but 42 minutes after the injury the rate began to rise and continued elevated until death, 70 minutes later.

It is interesting to note that, in one case (cat 10, group I), after crushing and massaging the muscles of the thigh, there was a gradual increase in the heart rate (196 to 212), while the fall in blood pressure was slight (122 to 100). Moreover, the blood pressure at this time could not be said to have reached a "shock level." Under the usual experimental conditions, we should expect to find with this slight drop in blood pressure either no change in the heart rate, or a slight fall. The fact that the heart rate was increased indicates the onset of a condition of shock, to which the blood pressure curve would have given no definite clue. This is in accord with the observations of Gesell (8) who noted that the development of shock might be indicated by the reduction in the volume flow of blood through the submaxillary gland as a result of vasoconstriction—when the drop in blood pressure had not yet assumed significant proportions.

It will be well, perhaps, to analyze briefly the individual experiments.

Group I. In which the rate was elevated during the development of shock. Five cases.

Cat 2, ♀, 2.9 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
1:35	38.1	143	228
1:40	38.1	152	228
1:45	38.1	155	228
1:45–48	Crushed muscles of left thigh		
1:48	38.1	69	228
2:00	38.1	118	237
2:05	38.2	94	248
2:15	38.1	84	242
2:25	37.6	79	234
2:35	37.8	64	231
2:45	38.0	52	220
2:50	37.2	48	214
3:00	37.8	41	224
3:05	38.0	40	222
3:10	38.1	40	221
3:15	38.0	24	202
3:25	37.9	20	184
Death of animal			

Average rate before injury, 228. Highest rise 20 beats, 17 minutes after injury. Rate elevated for 47 minutes, following injury.

Cat 5, ♀, 2.6 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:35	37.9	124	190
2:40	37.9	120	184
2:45	37.9	116	183
2:45–47	Crushed muscles of left thigh		
2:47	37.8	92	200
2:55	37.8	81	189
3:00	38.0	83	193
3:10	38.0	83	197
3:20	38.0	80	204
3:30	38.0	78	196
3.40	37.7	56	187
3:50	37.8	47	188
3:55	37.7	47	189
4:05	37.9	49	194
4:15	38.2	48	194
4:30	38.1	50	193

Average rate before injury 186. Highest rise 18 beats, 33 minutes after injury. Rate elevated for 43 minutes, following injury.

Cat 8, ♀, 3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:30	37.6	135	192
10:35	37.6	132	191
10:40	37.7	135	191
10:43–45	Crushed muscles of right thigh		
10:45	37.7	80	204
10:55	37.6	102	212
11:05	37.6	99	214
11:15	37.8	96	212
11:25	37.6	98	213
11:35	37.6	90	208
11:45	37.7	80	208
12:00	37.6	74	206
12:10	37.6	68	196
12:20	37.6	63	190
12:30	37.6	54	187
12:40	37.6	49	189
12:50	37.5	42	184
Death			

Average rate before injury 191. Highest rise 25 beats, 20 minutes after injury. Rate elevated for 85 minutes, following injury.

Cat 9, ♀, 3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Left sciatic and left femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:30	36.8	129	208
2:35	36.8	132	202
2:40	37.0	130	202
2:41–43	Crushed muscles of left thigh		
2:43	37.0	97	220
2:50	37.0	105	216
3:00	37.1	99	224
3:10	37.0	89	230
3:20	37.0	78	226
3:30	36.8	63	214
3:45	36.5	52	198
4:00	36.8	58	208
Death			

Average rate before injury 204. Highest rise 24 beats, 27 minutes after injury. In this animal there was a slight premortem rise, 10 beats above the rate of the previous observation.

Cat 10, 2.5 K. Ether anesthesia. Vagi cut in neck and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:50	37.8	140	196
10:55	37.8	140	198
11:00	37.8	140	196
11:03–05	Crushed muscles of right thigh		
11:05	37.8	96	198
11:10	37.8	135	198
11:20	38.0	122	196
11:24–25	Massaged muscles of crushed thigh		
11:25	38.0	112	204
11:30	38.0	120	200
11:35	38.0	115	208
11.45	38.1	108	212
11:50	38.1	105	212
12:00	38.1	100	208
12:10	38.0	88	214
12:20	37.9	82	212
12:25	37.8	80	210
12:40	37.6	83	208
12:50	37.7	77	198
1:00	37.7	74	194
1:15	37.9	45	204
Death			

Average rate before injury 197. Highest rise 17 beats, 65 minutes after injury. Rate elevated for 105 minutes, following injury. There was in this cat, as in cat 9, a slight premortem rise of 10 beats. In cat 9 this was associated with a slight rise in blood pressure, which was absent in cat 10. I can offer no logical explanation for this phenomenon.

Cat 1, group II, was the only case in which there did not appear to be any indication of over-activity of the adrenals shortly after injury. In this instance the pressure dropped after injury from an original level of 108 mm. to 65 mm., which is probably below the critical level for adequate nourishment of the heart. The recovery on the next reading was slight—74 mm. Hg., and thereafter the pressure never rose above this dangerous level. It is conceivable that the sharp and continued drop in pressure was injurious to cardiac tissue. Thus in a number of cases reported by Stewart and Rogoff (9, cases 443, 447, 448) a progressive fall of blood pressure below 80 mm. Hg. was attended by a more or less progressive fall in the heart rate. Similar observations have been made by Cannon and Smith. Probably in the presence of very low pressure

Group II. In which the rate was not elevated until shock was fully established. One case.

Cat 1, ♀, 2.4 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:20	37.8	105	194
10:25	37.8	109	190
10.30	37.8	108	190
10:30–33	Crushed muscles of left thigh		
10:33	37.8	65	212
10:40	37.8	74	193
10:45	37.8	73	186
10:55	37.8	72	175
11:00	37.4	68	178
11:05	37.4	63	182
11:10	37.4	62	183
11:15	37.7	56	188
11:25	38.0	51	198
11:35	38.2	53	206
11:50	38.4	54	203
12:05	38.1	54	209
12:15	37.9	38	204
12:20	37.8	36	210
12:25	37.8	38	216
Death			

epinephrin cannot have the degree of effect that it has when the pressure is not so much reduced. The harmful influence of a lessened blood flow is doubtless due both to oxygen want and to excess of carbon dioxide and perhaps other metabolites. Patterson (10) found a direct antagonism between the action of CO_2 and epinephrin on the rate of beat of the isolated heart—CO_2 slowing the rate, epinephrin making it more rapid. The combination of epinephrin and CO_2 had an effect that was somewhere between the effect of each separately, and in one experiment the rate was slower than the normal rate of the heart. In such a heart as we have in cat 1, we may conceive of asphyxia and the products of asphyxia as contending with an increased production of epinephrin for mastery over the heart, the one tending to decrease the rate of the heart, the other to increase it. It will be noted that no analogous condition—as regards blood pressure—was present in any of the five cases in which early rises in rate were observed. In cat 3, where there was no rise in rate, somewhat similar conditions prevailed.

Group III. In which the rate was not elevated during the development of shock. Three cases.

Cat 3, 4.1 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:35	37.0	147	189
10:40	37.0	147	189
10:45	37.0	130	193
10:50	37.0	133	192
10:55-59	Crushed muscles of left thigh		
10:59	37.0	60	186
11:10	37.0	63	192
11:15	37.1	75	187
11.20	37.2	76	190
11:25	37.2	77	190
11.30	37.2	75	182
11:35	37.2	57	176
11:40	37.2	49	173
11:50	37.2	39	174
12:00	37.1	25	172
12:05	37.1	15	150
Death			

Average rate before injury 191. Very slight change in rate for 26 minutes, then a drop of 16 beats. The blood pressure conditions in this cat were somewhat analogous to those of cat 1. In this case, however, the drop was greater—about 67 mm. Hg. The pressure was from the start at a dangerously low level after the injury, and it is possible that the heart had lost some of its power of reacting to epinephrin.

In the three cases of group III, there was no evidence of increased activity of the adrenal glands. In two of them (cats 12 and 13), I am not convinced that this can be adequately accounted for. It might be argued that were it not for increased secretion by the glands in these cases, the rate would have been much lower than was actually the case. It will be seen, however, on examination of the data obtained from control cats, whose adrenals had been removed before crushing the limb, that, save in one instance, there was no marked drop in the heart rate, in spite of the fact that the blood pressure was in comparable cases no lower than at corresponding points of the development of shock in cats 12 and 13, whose adrenals were intact.

Cat 12, 3.3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:20	35.7	128	172
2:25	35.7	133	168
2:30	35.7	136	168
2:35–37	Crushed muscles of right thigh		
2:37	35.7	85	168
2:45	35.8	94	172
2:50	35.8	89	172
2:55	35.8	85	172
3:05	35.7	74	172
3:15	35.7	63	168
3:25	35.4	59	164
3:35	35.4	57	162
3:45	35.3	56	160
4:05	35.8	59	166
4:20	35.8	59	164

There was practically no change in rate, despite a fair pressure for some time after the injury.

Cat 13, 3.5 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:40	38.0	127	196
2:45	38.0	123	196
2:50	38.0	122	196
2:52–53	Crushed muscles of right thigh		
2:53	37.9	100	192
3:00	38.2	86	194
3:05	38.3	84	192
3:10	38.4	81	196
3:15	38.5	77	200
3:25	38.4	74	200
3:35	38.2	71	200
3:45	38.2	71	196
4:00	38.1	50	190
4:15	38.1	44	184
Death			

There was a slight rise of temperature in this case. Allowing for this, the rate remained practically unchanged.

Group IV. In which the adrenal glands were removed prior to the induction of shock.

Cat 4. Ether anesthesia. Vagi cut and stellate ganglia removed. Adrenals removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
11:10	Adrenals out		
11:35	37.0	131	194
11:40	37.0	120	198
11:45	37.1	125	189
11:45–49	Crushed muscles of left thigh		
11:49	36.8	78	170
11:55	36.6	94	161
12:00	36.6	89	162
12:05	36.6	95	160
12:10	36.7	91	161
12:15	37.1	92	166
12:30	37.5	71	176
12:35	37.6	66	176
12:40	37.6	52	173
12:45	37.6	50	173
12:50	37.7	45	170
12:55	37.4	39	164
1:00	37.4	39	160
1:05	37.3	36	158
Death			

In this animal a fairly high initial rate was lowered at once about 16 beats after the muscles were crushed, and remained at about this low level during the development of shock. During the first 25 minutes after injury, the rate was, on the average, 27 beats below the original level.

Cat 6. 2.9 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Left sciatic and left femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:25	Right adrenal gland removed		
2:40	Left adrenal gland removed		
2:55	34.7	96	134
3:00	34.7	93	136
3:05	34.7	93	136
3:07–08	Crushed muscles of left thigh		
3:10	34.7	80	138
3:15	34.7	78	138
3:25	34.7	74	136
3:35	34.8	70	135
3:45	35.0	70	135
3:55	35.0	65	136
4:05	34.8	63	134
4:15	34.7	60	132
4.25	34.8	50	132
4:40	35.2	54	132
4:55	35.2	52	132
5:10	35.0	51	130

A very low original rate, with practically no change after injury.

Cat 7, 2.9 K. Vagi cut and stellate ganglia removed. Left sciatic and left femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:10	Left adrenal gland removed		
2:25	Right adrenal gland removed		
2:35	37.8	137	168
2:45	38.0	143	170
2:50	38.0	138	170
2:53–56	Crushed muscles of left thigh		
3:00	38.0	103	163
3:05	38.0	94	162
3:15	38.0	92	161
3:25	38.0	86	165
3:35	38.0	84	166
3:45	38.0	68	164
3:55	38.0	67	164
4:05	38.1	68	166
4:15	38.0	68	164
4:25	38.1	72	163
4:35	37.9	79	163
4:40	Gentle massage of crushed muscles		
4:45	38.3	70	160
4:55	38.1	69	156
5:10	37.9	65	156

Shock developed slowly in this animal, but there was no evidence of increased heart rate even when the blood pressure was above 90, after tissue injury.

Cat 14, 3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:00	Right adrenal gland removed		
2:15	Left adrenal gland removed		
2:30	37.0	118	164
2:35	37.0	116	163
2:40	37.0	118	166
2:42–43	Crushed muscles of right thigh		
2:43	37.0	81	164
2:55	37.1	100	152
3:05	37.0	100	154
3:15	37.0	97	158
3:25	37.0	90	154
3:35	37.0	84	154
3:45	37.1	80	156
4:00	37.0	78	154
4:10	36.8	76	156
4:20	36.9	70	152
4:30	37.0	65	150
4:40	37.0	60	146
4:50	37.0	54	144
5:00	36.8	46	143

In this cat, also, there was no rise in heart rate after crushing the thigh muscles, even when the blood pressure was above 90. The rate tended to be about 8 or 10 beats lower than the original rate for about 80 minutes after injury.

Of the control animals, cat 6 had what might be described as almost a basal rate. In other words, the possibilities of a drop of rate, as a result of the gradual decrease in blood pressure, were much reduced. It is probable that with the heart beating at this rate, its nutritional needs are not so great as in a faster beating heart doing just as much external work, and that it is not so susceptible to changes in blood supply below the usual critical pressure. In cats 7 and 14, also, the heart rate was lower than is usually the case in animals with adrenals intact. In cat 4, where the original rate was fairly high (average 193), tissue injury was followed by a marked drop in heart rate, associated with the lowered blood pressure, suggesting that the above hypothesis has a basis in fact.

Discussion. Of nine cases, there was in five a definite increase in the rate of the isolated heart with a falling blood pressure, early in the course of the development of traumatic shock. This increased rate appeared quickly, often in the first observation after crushing the thigh muscles. The continued elevation in rate can not be due to continued stimulation of nerve endings in the injured region, resulting in reflex discharge from the adrenals, for the change appears to as marked a degree in cases where the chief afferent paths above the region have been cut, as where they have not.

It must be emphasized that, while as a result of these experiments I take issue with Bedford when he states that there is an increase in epinephrin liberation only after a prolonged continuation of the conditions leading to shock, I am not prepared to deny his opinion that the quantity of epinephric material in the blood increases with the prolongation of the period of low blood pressure and shock, for the method I used has this limitation, that when the blood pressure becomes low—let us say 50–60 mm. Hg.—the tendency of the heart rate, as mentioned above is to drop, because the heart is being insufficiently nourished. Under these circumstances, the rate of the isolated heart would be the resultant of opposite factors, increased epinephrin liberation tending to accelerate it, and the decreased blood supply tending to make it slower. So, in the experiments described, it is possible that in the later stages of shock there was an increased discharge of epinephrin into the blood stream, which the method I used was unable to detect. Of course, the tendency of the heart rate to become slower as the pressure falls renders especially significant the actual *increase* of rate which was manifested in the foregoing experiments. In other words, the experimental conditions were opposed to the positive result which was obtained.

One case, of the remaining four, also showed an increase in heart rate after injury, but in this case the rise did not appear until shock was fully developed, as indicated by the blood pressure. This is the only case that gave results similar to Bedford's observations. I have already discussed a possible reason for the delay in the appearance of the rise in this case. It is remarkable that it should have appeared at all in view of the then long continued low pressure.

Relation of epinephrin liberation to the production of shock. If, in most cases, there is this increased liberation of epinephrin into the blood stream during the development of traumatic shock, the question arises as to its significance.

It has been postulated that shock is the result of exhaustion of the adrenal glands. The evidence for this is not convincing. Corbett (11) has stated that anything that depletes epinephrin favors the development of shock. His experiments, of which the details are omitted, tended to show that anesthesia, fright and trauma decrease the epinephrin content of the adrenal glands. Earlier, Bainbridge and Parkinson (12) and Parkinson (13) were unable to demonstrate chromaffin substance in the adrenal medulla in death from post-operative shock and other acute conditions, and asserted that in such acute conditions, associated with low blood pressure, the adrenal glands yield up their store of epinephrin. That this is a fact Short (14) has denied, his examination of adrenals after death from shock betraying no marked difference from controls.

But even assuming that the former observations are correct, they throw no light upon the production of shock, for they furnish no proof that depletion of the epinephrin store in the adrenals did not occur after shock had developed, rather than before.

Moreover, it is obvious that, according to an "exhaustion" hypothesis, even a normal secretion of epinephrin could not exist in the presence of shock. In the experiments which I report, increased activity of the adrenals may be observed in cases where shock was severe enough to be followed shortly by death, and where the blood pressure was at a dangerously low level. Thus, in cat 2 the rate was still elevated with the blood pressure at 64 mm., a drop of 86 mm. from the original level; in cat 8, at 68 mm., a drop of 66 mm.; and in cat 9, with the blood pressure at 63 mm., a drop of 67 mm.—definite evidence that shock was developing in association with an increased secretion of epinephrin.

But is this increased secretion, perhaps, the cause of the development of shock? Such a suggestion has been advanced. Thus, Bainbridge and Trevan (15) injected large amounts of epinephrin, keeping the blood

pressure at a high level, and Erlanger and Gasser (16) repeated these experiments, infusing into the femoral vein 6 to 11 cc. of a 1:1000 solution of adrenalin chloride for a period of 21 to 29 minutes. These two series of experiments gave similar results, namely, a marked drop in arterial pressure and symptoms of shock. But the conditions were sufficiently artificial to make them difficult of comparison with the actual conditions of traumatic shock in an animal whose supply of epinephrin is the result of his own manufacture.

In my experiments the highest increase of rate observed was no greater than 25 beats. In the course of recent experiments conducted by Cannon and myself, we have found that the denervated heart reacts quantitatively to the infusion of adrenalin chloride. The reaction is more exact when the animal is "reduced"—that is, when the carotid and subclavian arteries and the aorta below the renal arteries are tied off, and the mesenteric nerves are cut—but even when this procedure is not carried out, the increase of heart rate corresponding to the infusion of definite quantities of adrenalin chloride at a given rate may vary in different animals within comparatively narrow limits. Thus, in one experiment, adrenalin chloride, 1:100,000, injected at the rate of 0.002 mgm. per kilo per minute, produced a rise of 24 beats per minute in the heart rate; and in another, infusion of adrenalin at the rate of 0.0024 mgm. per kilo per minute resulted in a similar rise. Erlanger and Gasser, using dogs, injected very large amounts of adrenalin chloride, far more than would be secreted by the adrenal glands under any circumstances. In one experiment the rate was about 0.04 mgm. per kilo per minute, whereas, in my extreme case, the rate of production of epinephrin was probably in the neighborhood of 0.002 mgm. per kilo per minute. The rate of infusion by Erlanger and Gasser, therefore, can hardly be said to be analogous to the production of epinephrin in the body.

Moreover, Henderson, Prince and Haggard (17) infused, continuously or intermittently, 1:10,000 adrenalin chloride into the femoral vein at a rate of from 0.5 cc. to 1.0 cc. per minute, maintaining the blood pressure at a very high level for a period of from ½ hour to 2 hours, and then, on discontinuing the infusion, found that no symptoms of shock developed. There is, then, no real proof that an increased production of epinephrin is an essential factor in the development of shock.

That it is not an indispensable factor is obvious from the fact that traumatic shock can be produced in the absence of the adrenals or when they give no evidence of hyperactivity, other experimental conditions remaining the same as in such cases where the adrenals are overactive.

Cannon has emphasized the idea that in times of stress, hyper-secretion of the adrenal glands accompanies increased activity of the sympathetic division of the autonomic system. In the development of shock such increased sympathetic activity undoubtedly occurs, and one of its most important functions is to produce generalized vasoconstriction in non-vital regions, thus tending to confine the depleted circulation to the organs essential to life. It is possible to invoke a useful function for the over-production of epinephrin in the development of shock, rather than to suppose that the adrenals are running amuck, inducing shock either by hyper-secretion directly, or by exhaustion of their store of epinephrin. They are stimulated to over-activity very soon after the conditions that tend to result in shock are established, and it seems probable that this over-activity, when it occurs, acts as an accessory factor, aiding the sympathetic system in maintaining vasoconstriction, and so may be considered as an additional conservative mechanism, tending to protect the animal against the consequences of shock-inducing influences.

SUMMARY

1. In six out of nine cases reported, there is evidence of hyperactivity of the adrenal glands during the development of traumatic shock.

2. There is no sufficient reason to believe that either over-secretion of the adrenals, or their exhaustion, is a factor in the production of shock.

3. It is probable that over-activity of the glands, in the development of shock, is a conserving factor.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

IX. Further Evidence of Nervous Control of Thyroid Secretion[1]

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In the second paper (1) of this series was summarized the histological and anatomical evidence that the cells of the thyroid gland are innervated by non-medullated fibers arising in cervical sympathetic ganglia. Researches which revealed structural and chemical changes in the gland in consequence of stimulation or severance of nerves in the neck were also reported. Furthermore, we described experiments showing that excitation of the cervical sympathetic induces an action current in the thyroid—a result not seen after vagal stimulation. In the fourth paper (2) Levy presented confirmatory evidence of sympathetic control of thyroid secretion, as shown by increased sensitiveness of vascular responses to repeated standard injections of adrenalin.

The method used by Levy involved pithing the central nervous system to the mid-thorax, a procedure which, of course, excluded any tests of the effects of reflex or asphyxial discharge of sympathetic impulses. Another method permitting such tests was desirable. Since the adrenal medulla secretes continuously under experimental conditions, if the central nervous system is intact, it seemed that Levy's method might be reversed, i.e., that thyroid secretion could render more effective the continuously secreted adrenin. Moreover, it was thought that the heart, isolated from the central nervous system, would probably respond to the combined action of thyroid and adrenal substance. As shown in a previous paper (3), the denervated heart is remarkably stable in its performance and is unaffected by any but

[1] The results here reported were presented to the Society for Experimental Biology and Medicine, February 18, 1920. (See Proc. Soc. Exper. Biol. and Med., 1920, xvii, 88.) It is a pleasure to acknowledge here the support for the research obtained from a fund for thyroid investigation given by Dr. W. N. Bullard.

thermal changes and chemical agents brought to it in the blood stream. It manifests characteristic increments of rate when the adrenal medulla (3), (4) or the liver (5) is stimulated. Might it not show another typical change if a thyroid were added to the adrenal effect? Such was the idea at the start. Later, as will be described, it was proved that the thyroid can act directly, without requiring the presence of circulating adrenin.

It may appear strange that an attempt should be made to employ the same organ to test a number of different glands of internal secretion. The heart, however, is a representative muscular structure, continuously active, and working at a rate which is altered with alterations in the rate of metabolism, as, for example, when chemical changes are accelerated by heat (6). Some of the endocrine glands, especially the thyroid, have a pronounced effect on the metabolic rate of the body as a whole. If the heart as a representative organ discloses this effect and is responsive in a typical manner to thyroid stimulation, different from its response to stimulation of other glands, it can obviously serve for testing thyroid activity. And, compared with vascular reactions as a test, it has the advantage of not requiring a destruction of the upper portion of the central nervous system. With it, therefore, conditions known to evoke sympathetic impulses, e.g., afferent stimulation and asphyxia, can be tried, as well as direct excitation of the gland and of peripheral nerves.

Method. In this research the cat has been used for study. Our first experiments were performed under urethane anesthesia. The results were baffling; sometimes suggestive changes occurred, but often no definite effects were evident. Attention to other problems for a number of years had obscured the memory of earlier difficulties of the same kind. In 1916, Cannon and Cattell had reported that in studying the action current of the thyroid gland urethane was unsatisfactory as an anesthetic, and also that deep etherization was capable of abolishing effects that appeared readily when the anesthesia was not so profound (1, p. 62). When these facts were recalled, and light etherization was employed, we at once began to obtain consistent results under conditions to be described. By careful adjustment of the air and the ether vapor entering the trachea, it was usually possible to maintain for long periods a uniform anesthesia, as tested by the wink reflex. A return to urethane in two experiments after repeated successes with ether anesthesia revealed again the depressant influence of that drug.

Hoping that we might be able to dispense with anesthesia during the thyroid stimulation, we tried in several instances decerebration. This may prove to be a feasible method, but in the few experiments we tried the blood pressure fell to a low level and prevented a positive result, or the heart rate became so rapid that further acceleration was impossible. We confined ourselves, therefore, to the use of ether, given cautiously and carefully in order to reduce the element of excitement, and maintained at a low tension. In experiments on reflex stimulation the anesthesia was not so deep as to prevent dilatation of the pupil and retraction of the nictitating membrane while the stimulus was being applied.

The tracheal cannula was introduced as low in the neck and with as little disturbance to cervical structures as possible. The opening in the trachea, made at one side in order to avoid the ventral vein which drains the thyroid glands, was so small that after the cannula was pushed in a ligature was not needed to hold it in place.

In isolating the heart the first step, taken even before the trachea was opened, was the severance of both the vagus nerves and the cervical sympathetic strands low in the neck. The object of this early cutting of the sympathetic strands was to protect the thyroids as much as possible from any disturbance incident to operating on the stellate ganglia. To remove the stellates a small opening was made between the first and second ribs on either side, under artificial respiration, and while the ribs were separated by a rib spreader the ganglion was picked up in forceps, its nervous connections were cut with small scissors, and it was brought out entire. The ribs were then tied together (at full inflation of the lungs), the overlying muscles were sewed in two layers, and finally the skin opening was closed. After this operation was completed on each side the animal breathed normally, without artificial aid. Occasionally slight leakage, or failure of the lungs to inflate in all parts when the ribs were being tied, required withdrawing air from the pleural cavity. This was done through a hollow needle.

In isolating the heart from the central nervous system without disturbing the nervous connections of the thyroid gland, as was necessary in experiments on *reflex* and *asphyxial* stimulation, the nerves in the neck were not touched. The opening between the ribs, however, was much enlarged so as to permit a clear view of the nerves in the upper thorax. The typical arrangement of these nerves is shown diagrammatically in figure 1. On the right side the accelerator strands from the stellate ganglion join the vagus and pass down with it. On the left

side there is a trunk which passes directly toward the heart from the ganglion, and another which accompanies the vagus. It was our custom in operating for reflex and asphyxial stimulation to sever the vago-sympathetic trunk on the right, the vagus and the attendant trunk on the left, and also, very carefully, all fibers passing inward from the stellate ganglion on either side (at the points marked x, fig. 1) except that connecting the ganglion with the cervical sympathetic. Occasionally other nerve filaments were observed on the ventral side of the trachea near the heart; on the chance that these might be a source of

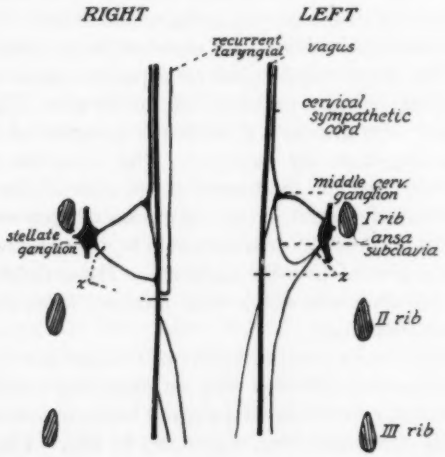

Fig. 1. Diagram showing the arrangement of the vagus nerves and the branches of the stellate ganglia in the lower neck and upper thorax of the cat. When the stellate ganglia were left in place the nerves were cut at the points marked x.

disturbance they also were cut. After thus doing all that was possible to isolate the heart without harm to the cervical sympathetic connections of the stellate ganglia, our standard of success was a fairly constant base line for the cardiac rate, and a return to that line after the rate had been disturbed by thyroid stimulation.

The effect produced by thyroid stimulation required often 3 hours and more for its completion. It is well known that the denervated heart is highly sensitive to alterations of temperature (7). Accordingly we were careful to maintain as nearly as possible a uniform temperature throughout the period of observation. The animal was laid on an

electric warming pad, the heat of which could be easily regulated. A sensitive thermometer in the rectum was examined every 10 minutes, and by properly adjusting the electric current the temperature of the animal was prevented from varying through more than a degree centigrade.

In the course of experimentation it soon became clear that positive results were seldom obtainable when the blood pressure was low, i.e., below about 70 mm. Hg. For example, on December 4, after a positive result had been obtained, and it had passed off, the blood pressure fell to 40 mm. Hg. when the adrenals were removed; thereupon a second attempt to obtain the result proved quite unsuccessful. On December 5, 8 and 9, after adrenalectomy, the pressure went down to about 50 mm. Hg., and the usual effect failed to appear. Again on December 22, after decerebration the pressure fell to 60 mm. Hg., and again there was failure. On January 2, with the pressure at 70 mm. Hg., the result was questionably positive. The negative results after adrenalectomy were not due to absence of the adrenal glands, as will be shown later. Since the heart is suffering from oxygen-want when the blood pressure falls below the critical level (8), it is probably unable to respond to the action of thyroid material. These failures should be compared with the successes which were obtained when the blood pressure was satisfactorily high.

To the difficulties with anesthesia, operation and low blood pressure was added an occasional difficulty with an initial high cardiac rate. In 20 cases the average rate of the denervated heart was about 200 beats per minute, with variations from about 180 to 220. These variations are in themselves of interest, but we have no explanation for them. Not very infrequently, however, the heart after being isolated from the nervous system would have a rate well over 240 beats per minute. Thus on January 6, an animal which had shown considerable excitement before and during anesthesia and which was actively digesting meat, had a pulse of 272 beats per minute, later falling to 240. On January 12, the rate after denervation of the heart (in a cat new to the laboratory) was 248, later 236. On January 29, the rate at the start was 252. When the heart was beating as rapidly as this we found that further attempts to accelerate it were futile. How to account for these occasional instances of very rapid pulse is not yet clear. It may be that the experience of the animal previous to anesthetization and operation was an important condition. For the present, however, we must leave the phenomenon unexplained. When such cases appeared, they were discarded as unserviceable.

The heart rate and blood pressure were registered by connecting with a mercury manometer the femoral artery (not the carotid because that would interfere with the circulation in the neck). Usually a record lasting 15 seconds was taken every 10 minutes during the period of observation.

The effect of thyroid massage. That a discharge of adrenin can be easily evoked by massage has been shown by several observers (9). It seemed possible that the thyroid also could be stimulated in the same way and would manifest a characteristic effect. Such stimulation has the advantage of being applied only to the gland that is being tested, and its effect limited to the region. The only other glandular structures which can be affected are the parathyroids, and if they are proved not to be influential, the result that occurs can only be attributed, mediately or immediately, to the action of the thyroid. This result can then serve as a standard by which to judge the results of exciting the thyroid by less direct ways.

That the rate of the denervated heart does not greatly vary if the animal, under uniform light ether anesthesia, is kept at a uniform temperature and its thyroid not stimulated, is shown in figures 2, 7 and 8. In the case represented in figure 2, the heart rates for an hour and a half before thyroid massage, registered every 10 minutes, varied only between 206 and 212 beats per minute. In that represented in figure 7 the variations for more than an hour lay between 196 and 204; and in that of figure 8 the range for an hour lay between 164 and 170.

In massage of the thyroid the gland was first laid bare and then stroked lengthwise with a smooth blunt dissector passed to and fro with slight pressure. This stroking was occasionally interrupted by gentle tapping. The massage rarely lasted more than 2 minutes. Never was it so vigorous as to cause any external bleeding or visible ecchymoses, though the gland might become slightly redder than before.

The effects of massage are shown in figures 2, 3, 4 and 5, and in table 1. As will be seen, the pulse is not much altered at first. Usually at the end of the 10-minute period following the stimulation, or in the next record thereafter, the rate is found to be accelerated. The acceleration rises to a maximum commonly in half or three-quarters of an hour; at this higher level the rate may remain for some time (in fig. 3, e.g., for about an hour). The increase has varied in our experiments from 16 to 50 beats per minute. After the maximum has been reached, and after the high level has been held for a considerable period, the rate gradually falls again to its former position. Two or 3 hours and more

may elapse before this restoration is complete (cf. figs. 3, 4 and 5 and also table 1). As shown in figure 3, massage of the other thyroid gland does not then reproduce the first effect. This may be merely

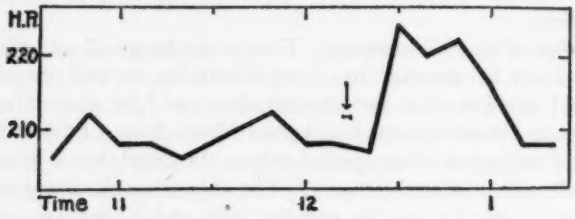

Fig 2. Graph showing absence of noteworthy variations of the heart rate during 100 minutes (10:40 to 12:20), and then a rise from 208 to 224 beats per minute, following massage of the right thyroid gland for 2 minutes (12:13–15).

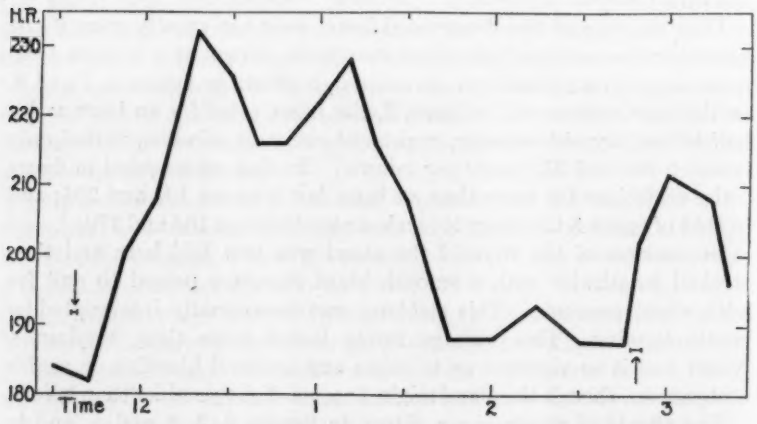

Fig. 3. Graph showing rise of heart rate from 182 to 232 beats per minute and persistent high rate for about 1 hour, induced by massage of the right thyroid gland for 2 minutes (11:36–38). Subsequent massage of the left thyroid for 3 minutes (2:47–50) caused a minor acceleration, from 188 to 212 beats per minute.

because the first effect has already occurred, but we have the impression that mere delay under ether and under the circumstances of operation renders the massage less capable of inducing a faster rate (cf. e.g., fig. 2).

That the faster rate induced by massage of the thyroid is not in fact due to disturbance of the parathyroids was proved by excising aseptically the one which is embedded on either side and later manipulating the thyroid alone. The effects were such as described above.

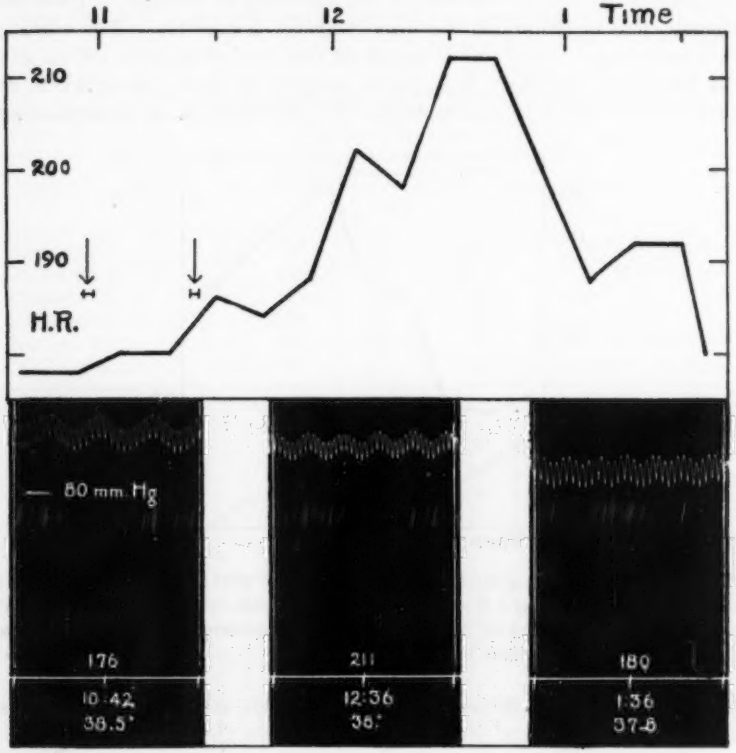

Fig. 4. Graph with samples of the original record showing absence of effect after massage of the right submaxillary gland (10:56–58), and increase of heart rate from 180 (11:18) to 211 beats per minute (12:36) after massage of both thyroid glands (11:23–25). The numbers above the line (zero blood pressure; 5-second intervals) here and in other similar records represent the heart rates per minute; the figures below, the time and the rectal temperature.

It might be supposed that other organs would share with the thyroid the capacity to accelerate the heart. Massage of the adrenal gland and also of the liver (5) can indeed have that effect, but it occurs at

once after the manipulation and passes away within a few minutes at the outside—i.e., before the *latent* period of the thyroid would be ended. In order to test another glandular structure, the submaxillary gland was vigorously stroked, pressed and tapped upon for 2 minutes. As shown in figure 4, no change resulted in the following 25 minutes. Then the thyroid was massaged in the usual way for 2 minutes. The cardiac rate soon began to rise and in about an hour had risen from 180 to 211 (31 beats) per minute. No greater amount of tissue disturbance, as such, was involved in manipulating the thyroid than in manipulating

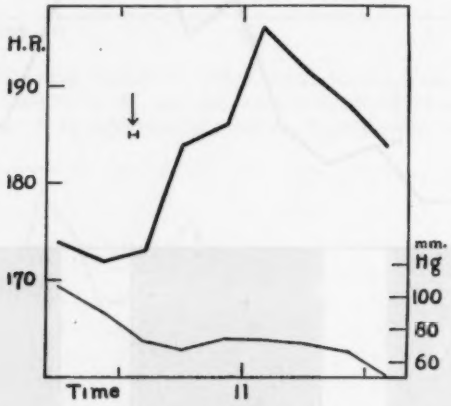

Fig. 5. Graph showing cardiac acceleration (from 172 to 196 beats per minute) after massage of the right thyroid gland for 2 minutes (10:32–34). The adrenal glands had been removed (at 9:35). As the blood pressure (lower line) fell below 70 mm. Hg., the experiment was interrupted.

the submaxillary, if, indeed, there was as much, and yet the effect was altogether different and characteristic when the thyroid was disturbed.

A question which early arose was whether the cardiac acceleration was due to the direct influence of thyroid substance carried to the heart, or was indirectly caused by thyroid stimulation of the adrenal medulla. As already remarked, the blood pressure was low in a number of experiments in which the adrenals were removed after denervation of the heart, and in these instances thyroid massage was ineffective. That this failure does not indicate that the adrenal glands are essential to the occurrence of a faster beat is shown in figure 5. Unfortunately about an hour after the stimulation the blood pressure fell below 70 mm.

Hg. and the experiment was therefore discontinued. The characteristic positive effect produced by thyroid massage in this and another instance, however, proves that the acceleration of the pulse is not mediated through adrenal secretion.

A single attempt to secure a faster heart rate by thyroid stimulation when the cardiac nerves were intact led to a questionable result. The

TABLE 1

Acceleration of the denervated heart by stimulation of the thyroid gland

DATE	HEART BEATS PER MINUTE			DURATION OF FASTER RATE
	Initial	Maximal	Increase	
Massage				
December 3	182	232	50	2 hours 40 minutes
December 4	208	224	16	40 minutes
December 6	124	150	26	3 hours
December 17	188	212	24	2 hours 10 minutes
January 10	204	220	16	50 minutes
February 4	180	211	31	2 hours 10 minutes
Cervical sympathetic stimulation				
December 13	192	220	28	3 hours 20 minutes
December 23	220	244	24	2 hours
January 5	144	172	28	3 hours 30 minutes
January 6	200	222	22	2 hours 10 minutes
Reflex stimulation				
January 13	176	208	32	3 hours +
January 22	212	240	28	2 hours
January 28	192	226	34	2 hours
Asphyxial stimulation				
January 31	198	216	18	1 hour 45 minutes
February 10	156	204	48	2 hours 30 minutes +
February 11	183	207	24	2 hours 20 minutes

heart rate which for 24 minutes had varied only between 190 and 192 beats per minute began to rise shortly after massage of the thyroid (12:42–45) and continued rising gradually until 1:42 when the rate was 212; there it remained for about 15 minutes, and then slowly returned to 200 beats per minute, at 2:30. The rise and fall were typical, and during most of the period the etherization was unchanged.

Before the thyroid stimulation, however, some vomiting movements (at 11:35) had sent the heart rate through the same range (192 to 212), with a return to 192 at 12:12. During this period the etherization was uniform. These incidental variations of rate when the nervous connections of the heart are intact render conclusions uncertain. We therefore relied on the denervated heart.

The effect of cervical sympathetic stimulation. In stimulating the cervical sympathetic we applied a mechanically interrupted tetanizing current to the strand in the neck in some instances and as it left the stellate ganglion in others. In different experiments the stimulation lasted from 5 to 15 minutes. The current was so weak that the iris was only about three-fourths withdrawn and was oscillating throughout the period as the stimulus went on and off.

The results are illustrated in figures 6 and 7 and in table 1. It will be seen that the heart is accelerated much as it is when the thyroid gland is directly massaged, and that the acceleration lasts for comparable periods.

It seemed possible that sympathetic impulses might affect other endocrine organs (e.g., the anterior lobe of the pituitary body) and that consequently the effect produced might be complicated by other than thyroid substance. In order to test this possibility we removed the thyroid on one side. We attempted to do this at the beginning of the experiment, but were confronted with a heart rate of 228 beats per minute, which in the course of 3 hours gradually fell to 180 beats. This might have been due to liberation of thyroid material during the removal of the gland. In order to avoid any possible thyroid effect except that in which we were interested, we removed the gland aseptically the day before the experiment. As shown in figure 7, stimulation (for 10 minutes—9:50–10:00) of the cervical sympathetic branch from the stellate on the side from which the thyroid had been removed had no effect. The nervous connections were intact, for the nictitating membrane was withdrawn and the iris was oscillating while the stimulus was intermittently applied. The same stimulation (for the same period—10:29–39) of the corresponding branch on the other side of the neck, where the thyroid was present, induced a typical increase of the heart rate. Within 10 minutes the pulse had begun to rise from the initial level of 200 beats and after 50 minutes it had slowly risen to 222. Thereupon the rate began to descend; in an hour and 10 minutes it had returned nearly to its initial level. The correspondence between the effects of thyroid massage and the effects of cervical sympathetic

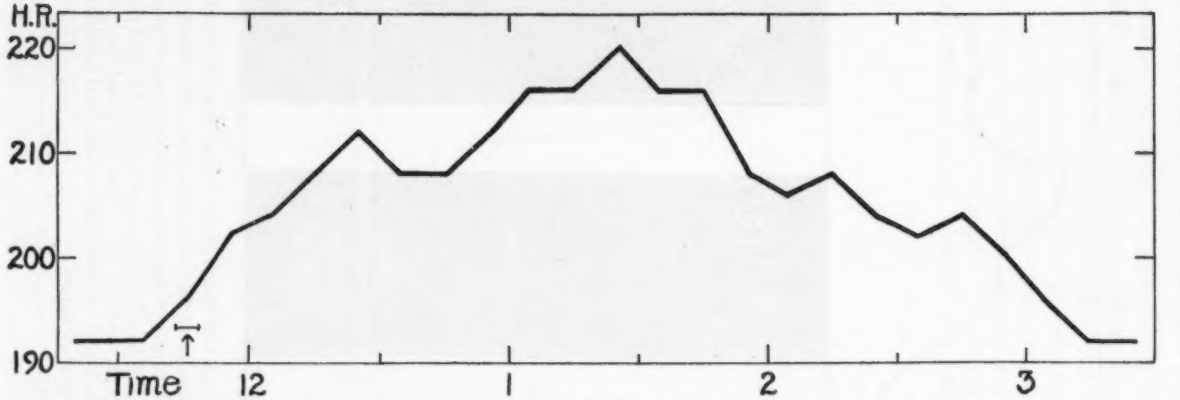

Fig. 6. Graph showing cardiac acceleration from 192 to 220 beats per minute, following stimulation of the right cervical sympathetic strand (11:43–48). The stimulus was a weak tetanizing current which caused a slight dilatation of the pupil and a slight retraction of the nictitating membrane.

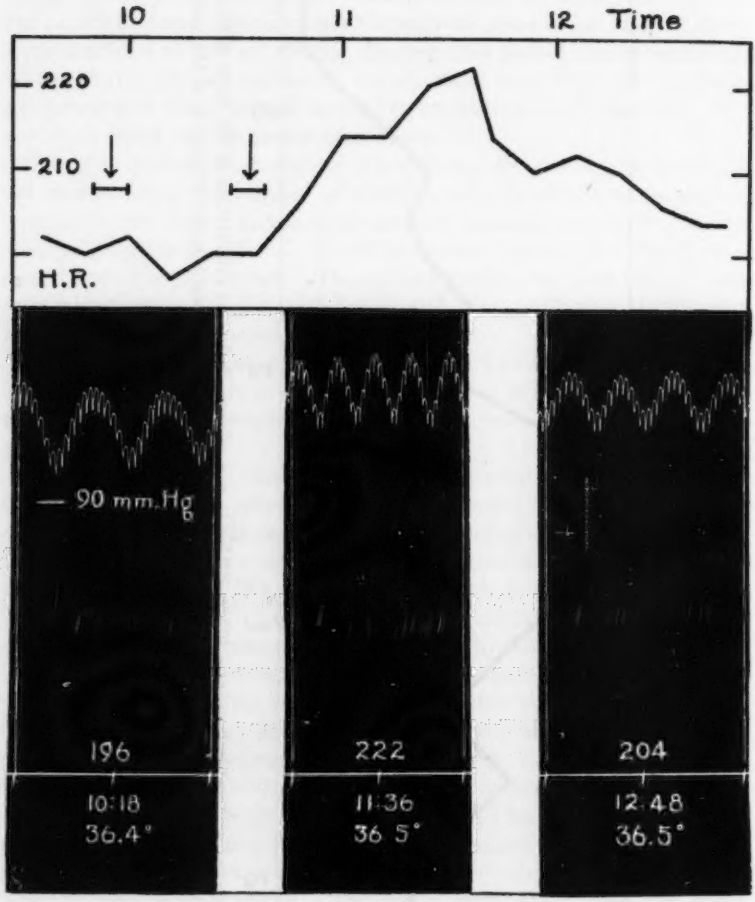

Fig. 7. Graph with samples of the original record showing absence of effect when the cervical sympathetic strand was stimulated (9:50–10:00) on the left side from which the thyroid gland had been removed, and a typical cardiac acceleration (from 200, at 10:24, to 222 beats per minute at 11:36) when the strand was stimulated (10:29–39) on the right side where the gland was present. The stimulus was in each instance a mechanically interrupted tetanizing current, just adequate to keep the iris oscillating.

stimulation when the thyroid is present (cf. figs. 4 and 7), and the absence of any influence of sympathetic stimulation when the thyroid is lacking seems to us to justify the conclusion that the faster cardiac rate is due to thyroid secretion induced by sympathetic impulses.

The effect of afferent stimulation and asphyxia. The rise of blood pressure which can be brought about by stimulation of a sensory nerve or by asphyxia is a manifestation of the efficacy of these measures in evoking sympathetic activity. Elsewhere evidence has been adduced

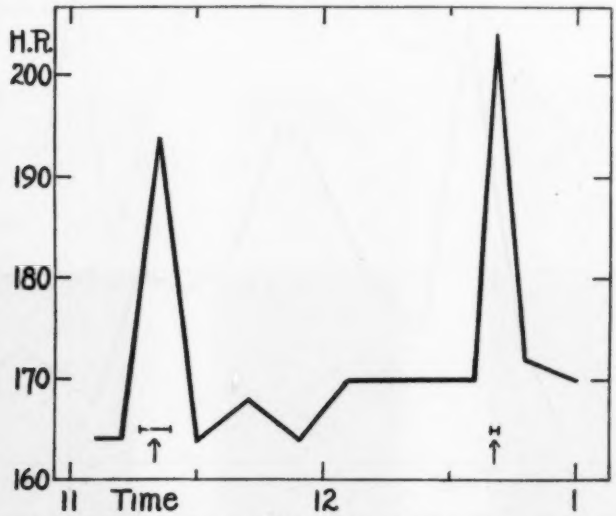

Fig. 8. Graph showing quick rise and fall of heart rate in consequence of afferent stimulation (sciatic nerve, 11:18–25) sufficient to cause slight dilatation of the pupil, and of asphyxia (12:40) for 105 seconds. *Both thyroid glands had been removed;* the accelerations are due to adrenal and probably also to hepatic secretion.

that these modes of exciting the sympathetic will provoke a secretion from the adrenal medulla, and possibly also from the liver, as revealed by a faster pulse (4), (5). The influence of these glands, therefore, would appear in any experiment demonstrating a reflex or asphyxial secretion from the thyroid. In order to have a background for judging the thyroid element in the response, as distinct from the adrenal and hepatic elements, we removed both thyroid glands aseptically and the next day recorded the change of rate of the denervated heart in response to afferent stimulation and asphyxia. The results are given in figure 8.

Interrupted tetanization of the sciatic nerve for 7 minutes (causing slight dilatation of the pupil) increased the pulse from 164 to 194 beats per minute. The heart promptly returned to its previous rate. There it remained during the following hour, varying only between 164 and 170. Asphyxia for 105 seconds then sent up the rate from 170 to 204 beats per minute; and again the heart promptly resumed its former pace, maintaining it thereafter without considerable change. It is not necessary now to discuss further the occasion for this cardiac accel-

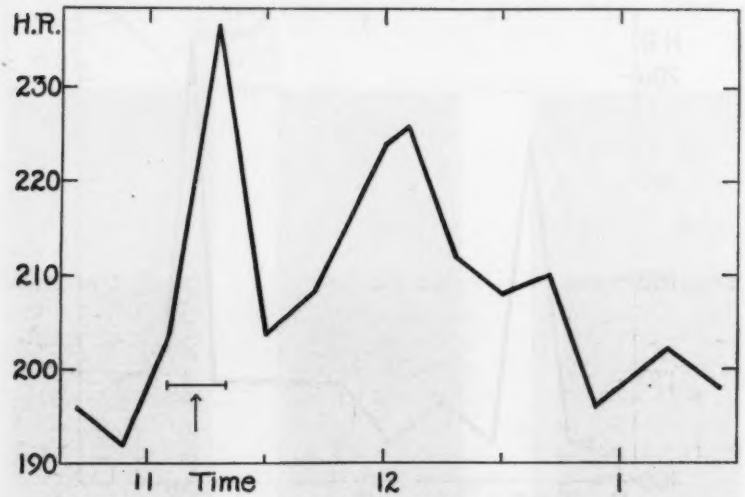

Fig. 9. Graph showing the quick rise and fall of rate shown in figure 8, followed by the slow rise and fall typical of thyroid stimulation. Both thyroid glands were present. The cardiac accelerations (from 192 to 236 and later to 226 beats per minute) were due to stimulation of the right brachial nerve with an interrupted tetanizing current from 11:05 to 11:20; only during the last 4 minutes were the pupils widened and the nictitating membranes withdrawn.

eration. As stated above, material discharged into the blood stream from the adrenal medulla and the liver accounts for the quick rise and fall of the heart rate in consequence of afferent stimulation and asphyxia. On the other hand, when these two modes of arousing sympathetic nerve impulses are applied when the thyroid glands are present, they give rise to characteristic additional changes of heart rate, resembling those already described as occurring after thyroid massage or after stimulation of the gland through the cervical sympathetic strand. These effects are shown in table 1 and also in figures 9 and 10.

In the experiment illustrated by figure 9 an interrupted tetanizing current (3 seconds on and 3 seconds off) was applied to the brachial nerve for 15 minutes. Only during the last 3 or 4 minutes was the anesthesia reduced to a degree that permitted reflex withdrawal of the

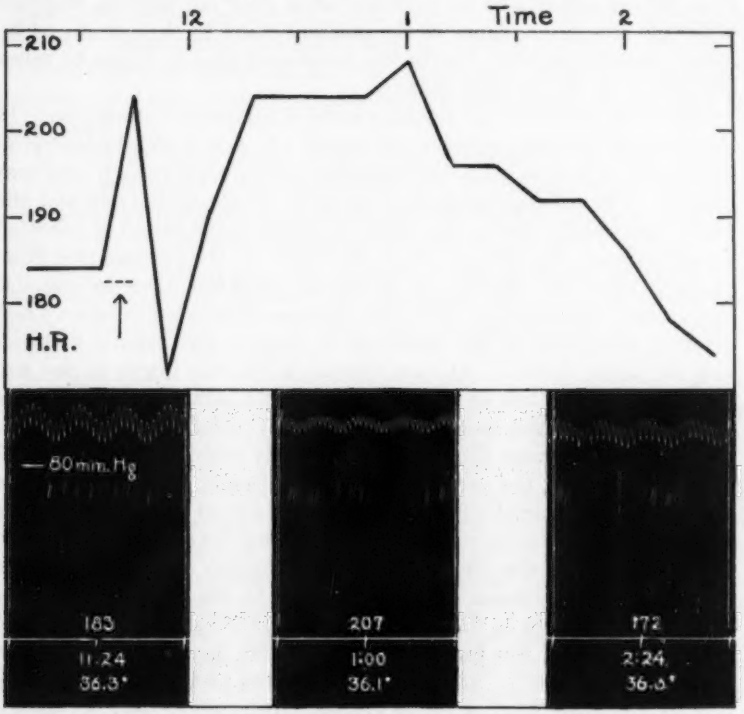

Fig. 10. Graph with samples of the original record showing the quick rise and fall of heart rate shown in figure 8, followed by the slow rise and fall typical of thyroid stimulation. Both thyroid glands were present. The cardiac accelerations (from 183 to 204 and from 172 to 207 beats per minute) were due to repeated periods of asphyxia, six in all, from 11:32 to 11:48, each lasting 1.5 or 2 minutes. The right nictitating membrane was completely withdrawn, the left nearly so, during the asphyxiation.

nictitating membranes and dilatation of the pupils. As the figure shows, the first effect was the typical sharp increase of rate (from 192 to 232 beats per minute) due to reflex adrenal (and hepatic?) secretion, and this was followed by the rapid fall shortly after the stimulation ceased.

Almost at once, however, the rate began to rise again and continued to rise in the slow manner characteristic of thyroid influence, until a maximum (226 beats per minute) was reached, and then slowly subsided to the former level. The absence of this second response when the thyroids are absent and its presence when they are present, together with the close resemblance of the positive effect to that produced by direct thyroid massage, justify the conclusion that it is due to reflex excitation of the thyroid glands.

Figure 10 illustrates the similar results of asphyxial stimulation. In this case the tracheal cannula was closed 1.5, 2, 2, 2 and 2 minutes in succession, with a period of breathing for 1 minute before each recurrent closure. The nictitating membranes were wholly withdrawn and the right pupil widely dilated (the left less so) during the period of repeated asphyxiation. Again there was a rapid rise of the cardiac rate from 183 to 204 beats per minute and an equally rapid fall when the stimulation ceased, a duplication of the first changes illustrated in figure 8 and properly ascribable to the discharge of adrenal and hepatic material into the blood stream. At once thereafter the rate began to rise and after a little more than an hour it had reached a maximum of 207 beats per minute. Another hour passed before the initial rate was restored.

In some of our cases the heart rate fell only slightly after the initial adrenal effect, i.e., the secondary thyroid influence very soon began to appear. On January 13, for example, the rate went up from 176 to 208 beats per minute during sciatic stimulation, and it had returned only to 192 when the fall was checked and the secondary rise began; it continued until a maximum (208 beats) equal to the adrenal effect was attained. Similarly in the asphyxial series (Feb. 10) asphyxia caused an acceleration of the heart from 156 to 232 beats per minute (an increase of 76 beats!). The rate dropped down to 194 and then slowly rose to 204 before starting on its gradual downward course.

General considerations. In contribution IV of this series Levy stated that though in his experiments adrenalin became more effective as a pressor agent after thyroid stimulation it did not become more effective as an accelerator of the heart. It is noteworthy, however, that in one case cited by him (2, p. 498) the basal rate of the heart increased 15 beats per minute after excitation of the left cervical sympathetic strand. This may have been an instance similar to those described in the foregoing pages, though the absence of a temperature record does not permit a definite conclusion to be drawn from his data. Certain it is that our present results confirm those of Levy and the earlier work of

Cannon and Cattell (1) in showing that the thyroid is capable of exerting a fairly prompt action and that the gland is subject to sympathetic control. The maximal heart rate in our experiments appeared sooner (in 30 to 45 minutes) after the stimulation than did Levy's maximal pressor effect of adrenalin (in 2 to 3 hours). This discrepancy may be related to a difference of action of thyroid material on the two structures —vessel wall and cardiac muscle—or it may be due to the very low blood pressure in Levy's pithed animals and the consequent retarding of all responses.

In an earlier paper of this series the results reported by Rahe, Rogers, Fawcett and Beebe (11) and by Watts (12), indicating that sympathetic stimulation reduces the iodine content of the thyroid on the side stimulated, were mentioned as supporting the view that the gland is subject to sympathetic impulses (1). Recently Van Dyke has repeated this work and has found that the variations in the iodine content on the two sides, stimulated and control, lie within the range of differences normally found in unstimulated animals (13). We know too little about either the character or the effective amount of the material given off by the gland to permit us to judge the significance of variations or the absence of variations in the iodine content. Even if Van Dyke's results are confirmed, the absence of chemical differences beyond those normally present in the two thyroid lobes could not be interpreted as contradicting the positive evidence of physiological effects of thyroid stimulation such as have been described in the foregoing pages.

In discussing sympathetic control of the gland Van Dyke mentions a number of investigators—Burget (14), Marine, Rogoff and Stewart (15), and Troell (16)—who have failed to obtain any effects on uniting the phrenic nerve with the cervical sympathetic strand, and he associates these failures with his own results as throwing doubt on sympathetic control of thyroid secretion. These failures, however, need not now concern us; they will be considered in a later paper when the positive experiments are described in detail; for the present we wish only to note that they have little or no bearing on the results we are now reporting.

In conclusion we wish to emphasize once more the importance of using the sympathetic fibers themselves rather than the superior or recurrent laryngeal nerves as means of testing the nervous government of the thyroid apparatus. Stimulation of the laryngeal nerves may induce effects such as we have obtained—indeed, we have seen acceleration of the denervated heart after exciting the superior laryngeal

branch of the vagus—but that does not prove that vagus impulses influence the gland. As pointed out in a previous paper (1), these *branches* of the vagus may have an admixture of sympathetic fibers and therefore the results of stimulating them are not decisive as to the division of the autonomic system that is effective. Our data in this and in previous communications show that it is the sympathetic division that acts—a fact of much importance in interpreting the function of the thyroid gland in the bodily economy.

SUMMARY

Gentle massage of the thyroid gland in the cat for 2 or 3 minutes will cause an increased rate of the denervated heart amounting in some instances to 25 per cent over the basal rate. The development of the maximal increase of rate is usually slow, requiring from 30 to 60 minutes and passing off in a similarly slow manner (see figs. 2, 3 and 4).

Massage of another gland, e.g., the submaxillary, does not cause this effect (see fig. 4).

The augmentation of heart rate caused by thyroid massage occurs in the absence of the adrenal glands (see fig. 5).

Stimulation of the cervical sympathetic trunk as it leaves the stellate ganglion induces a similar augmentation of the rate of the denervated heart; this does not occur if the thyroid gland has previously been removed (see figs. 6 and 7).

If the thyroid glands have been previously removed, sensory stimulation and asphyxia induce only the brief increase of rate due to adrenal and hepatic discharge (see fig. 8).

If the cardiac fibers from the stellate ganglia are severed, as well as the vagus nerves, and an afferent nerve, such as the sciatic or brachial, is stimulated under a degree of anesthesia which will permit reflex retraction of the nictitating membrane and dilatation of the pupil, there is a primary increase of rate due to adrenal secretion, followed by the slowly developing increase characteristic of the thyroid effect (see fig. 9).

If the vagi and the cardiac fibers of the stellate are cut, and the animal is asphyxiated under conditions which permit the eye changes described above, there is a similar primary rise due to adrenal secretion, followed by the secondary thyroid effect (see fig. 10).

Addendum. Attempts to repeat these observations in March and April have resulted in slight or negative effects. These rather striking differences are probably correlated with the remarkable seasonal variations in the iodine content of the thyroid gland reported by Seidell and Fenger (Journ. Biol. Chem., 1912, xiii, 523, Bulletin U. S. Hygienic Laboratory, No. 96, 1914, p. 67). These observers found that the percentage of iodine in the dried thyroid in March, though averaging one third, may be less than one eighth the percentage found in the late summer and autumn months.

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THE EFFECT OF SOME POLYHYDRIC ALCOHOLS ON THE BEHAVIOR OF RATS IN THE CIRCULAR MAZE

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In a preceding paper the authors have described their studies of the comparative effects of ethanol, caffeine and nicotine on the behavior of white rats in the circular maze (1). It was pointed out that this method of study is well adapted to the investigation of the narcotic properties of various drugs, as the maze enables one to determine even slight depression of the central nervous system and impairments of the neuro-muscular mechanism. Following the study of ethanol in this connection it was deemed desirable to inquire into the narcotic properties of small doses of other alcohols. It is well known that the higher mono-acid alcohols of the fatty acid series become more toxic with the increase in their molecular weight, following what is known as Richardson's law (2). A comparative study of primary and secondary alcohols carried out by one of the authors has also shown that while the secondary alcohols are less toxic than the primary ones, nevertheless the toxicity of analogous members increases as one goes up the series (3). It was therefore deemed useless to study the narcotic properties of the higher mono-acid alcohols. On the other hand the toxicity of some polyhydric alcohols is known to be not very great and a study was therefore undertaken to determine whether such alcohols are capable of depressing the brain and neuro-muscular mechanism of rats or, in other words, whether they exert a narcotic effect.

In the present investigation a number of such polyhydric alcohols was studied after injection intraperitoneally in white rats. The method of study was the same as described in the previous paper. Albino rats were trained to solve the maze problem so as to find their way through labrynthian paths to the center of the maze without committing any errors and in the shortest period of time possible. After the animals had been trained the various drugs to be studied were administered and the behavior as well as various somatic changes were noted at various intervals after the injection of the drugs.

Experimental data. A total of 40 rats was employed in this investigation. Most of the animals were male but a few experiments were also made on female rats. The animals at the beginning of the investigation were on the average about 45 days of age and in perfect physical condition.

The following polyhydric alcohols were studied: ethylene glycol $C_2H_4(OH)_2$, glycerol $C_3H_5(OH)_3$, erythrite, $C_4H_6(OH)_4$, arabite $C_5H_7(OH)_5$ mannite $C_6H_8(OH)_6$, dulcite $C_6H_8(OH)_6$, perseite $C_7H_9(OH)_7$ and volemite $C_7H_9(OH)_7$. Of these the first two are liquids while the others are crystalline solids. The drugs were dissolved in water, in concentrations of from 1 to $3\frac{1}{2}$ per cent. Stronger solutions were not made in order to avoid mechanical irritation due to the salt action or the physical phenomenon of osmosis, etc. Control experiments were

TABLE 1

POLYHYDRIC ALCOHOLS	MINIMAL EFFECTIVE DOSE PER 100 GRAMS OF RAT
	mgm.
Ethanol C_2H_5OH	80
Glycol $C_2H_4(OH)_2$	120
Glycerol $C_3H_5(OH)_3$	160
Erythrite $C_4H_6(OH)_4$	290
Arabite $C_5H_7(OH)_5$	230
Mannite $C_6H_8(OH)_6$	320
Dulcite $C_6H_8(OH)_6$	120
Perseite $C_7H_9(OH)_7$	over 380
Volemite $C_7H_9(OH)_7$	over 380

made with injections of normal or physiological sodium chloride solutions, in order to determine the effect of injection of large volumes of fluid. It may be stated at once that injections of even 10 or 12 cc. of normal saline solutions produce very little or no effect on the rats half an hour after injection.

Results. The results obtained with the various polyhydric alcohols are expressed in the subjoined table. The object of the experiments was to determine the smallest quantity of the drugs used which produced a depression in the behavior of the rats. The average minimal effective doses in this respect are expressed in the table. It will be noted that glycol is quite depressant or, using the term in its broadest sense, "narcotic" for the rats, a dose of 120 mgm. per 100 grams of rat being sufficient to produce a depression in the behavior of the animals.

An excitement stage was not noted after any doses of the drug, as indicated by the method used.

The effect of glycerol or glycerine, the tri-acid alcohol, was also extremely interesting. The average minimal effective dose of glycerol was found to be about twice as great as that of ethyl alcohol, or 160 mgm. The higher members of the series were also found to produce depression provided a sufficient dose was administered. The tetra-hydric alcohol, erythrite, required 290 mgm. per 100 grams of weight to depress the animals. The pentra-hydric alcohol, arabite, required 230 mgm., while the two hexa-hydric alcohols studied, namely mannite and dulcite, required 320 mgm. and 120 mgm. respectively. It is interesting to note that the two isomers, mannite and dulcite, differed in their toxicity. The authors, however, did not pursue any further inquiries concerning the relative toxicity of various isomers of the various higher alcohols studied. Two members of the hepta-hydric alcohols were examined. These were the rare substances, perseite and volemite. These compounds, however, were found to be very little toxic, so that comparatively very large volumes of these solutions had to be employed and the results obtained while indicating a narcosis or depression were not entirely satisfactory as the depression might have been due to their salt action.

Discussion. It is evident from the above table that all of the substances examined produced a depression of the neuro-muscular system and are "narcotic" in the broad sense of the word. Of especial interest is the action of the first two members of the series; namely glycol and glycerol. Poison cases following the ingestion of glycol and glycerol are *not* unknown. Symptoms of intoxication in animals have been described by Dujardin, Baumetz and Audige (4) and others. References to intoxication following the ingestion of large quantities of glycerine have been recorded by Kobert (5) and Kunkel (6). In the present investigation no excitement stage was noted after any of the drugs, even after small doses. It is interesting to note that while the potency of the drugs examined generally decreases with their molecular weight, exceptions occur as noted in the case of erythrite and arabite on the one hand and the isomers, mannite and dulcite, on the other. When one, however, compares the toxicity of the various drugs examined in their relation to their molar solutions the difference in toxicity is not so striking.

SUMMARY

1. A number of polyhydric alcohols was studied on rats in the circular maze.

2. It was found that all of the polyhydric alcohols when administered in sufficient quantity produced a depressant effect as indicated by this method.

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A COMPARISON OF WAVES OF BLOOD PRESSURE PRODUCED BY SLOW AND RAPID BREATHING

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It has long been known that, synchronously with the phases of normal respiration, changes in blood pressure occur. In continuous blood pressure records these changes appear as waves. Each wave is completed during a single respiration, the rise and fall of pressure having a definite relation to inspiration and expiration (fig. 1). The object of this research was to compare these effects of respiration with changes in blood pressure produced by respiration of a rapid rate, using as a basis of study the observation that respiration, approximating closely the rate of heart beat, elicits long oscillations of blood pressure resembling those described above. These waves were observed by one of us in sacrifice experiments on the dog and cat in which for some unknown cause the respiration became markedly accelerated. Analysis of such waves shows that the series of heart beats associated with one complete oscillation of pressure is composed of one beat less (fig. 2) or one beat more (fig. 3) than the number of respirations occurring in the same interval of time. A rough analogy may be made between the cardio-respiratory waves of blood pressure produced under these conditions and the alternate reinforcement and interference of sound that occurs when two tuning forks of slightly different frequencies are made to vibrate simultaneously. We, therefore, refer to these oscillations as *cardio-respiratory interference* waves to distinguish them from what we designate as *simple cardio-respiratory* waves, in which the relation of respiration to heart rate is always that of one respiration to several heart beats.[1]

Knowing the nature of the cardio-respiratory interference waves, and the advantages (1) which the interference method offers for

[1] While the changes in pressure in the simple waves are in effect due to interference and augmentation by respiration, yet the interference is quite different from that referred to in the physical interference of sound. Bearing this in mind we feel that the names we have used will serve to differentiate the two main types.

investigation, the opportunity of producing these waves voluntarily in man was used to obtain a more extended series of blood pressure waves than the fortuitous experiments on the dog and cat would permit. The

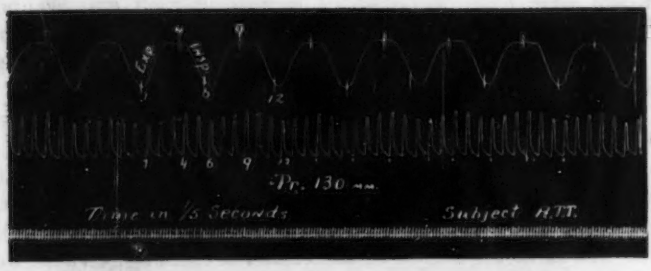

Fig. 1

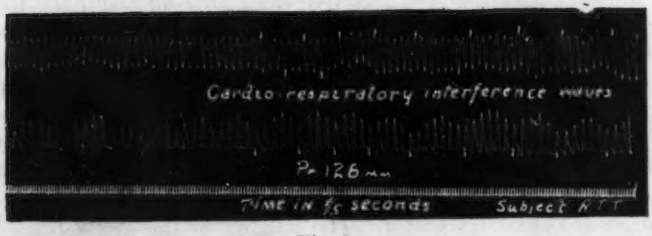

Fig. 2

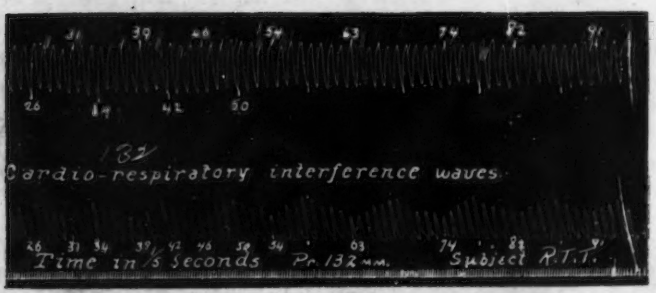

Fig. 3

experiments on man, therefore, supply the bulk of the data for this paper; and the experiments on the dog and cat are supplementary

Method. Simultaneous tracings of blood pressure, respirato ments, and time in seconds or fifths of seconds, were re

smoked paper by means of a long-roll kymographion revolving at a speed suitable for accurate measurements (figs. 4 and 5), the subjects being physiology students between the ages of 20 and 30 years, most of whom were men. Satisfactory records in each case comprised: *a*, simple cardio-respiratory waves; *b*, cardio-respiratory interference waves produced under two conditions—*1*, with respiration slightly faster than the heart rate, and *2*, with respiration slightly slower than the heart rate. Complete data which are summarized in figure 17 were obtained from thirty-two individuals.

Respiration was traced by means of a recording tambour suitably connected with a pneumograph made of a short metal cylinder, the ends being closed with rubber dam. The pneumograph was adjusted to the thorax in the region of the ensiform cartilage. There were a few exceptions to this procedure notably, in the case of women, where the adjustment of the pneumograph was higher up about the thorax. The subject was instructed to breathe synchronously with a metronome set to oscillate at the desired rate. For the simple cardio-respiratory waves the subject was instructed to breathe slowly and comfortably deeply, the metronome in most cases being dispensed with. No effort was made to have the respiration conform to any type such as "abdominal" or "thoracic."

Continuous blood pressure records were obtained by means of an Erlanger sphygmomanometer. The validity of the method has been demonstrated by Erlanger and Festerling (2) and their procedure for showing the effect of respiration on blood pressure has been largely followed in these experiments. The pressure in the cuff is maintained at a point slightly below systolic level[2] since it is at this pressure, according to Erlanger (2), that changes in the intra-arterial pressure give the greatest changes in the excursions of the writing lever. Under these conditions a rise in the intra-arterial pressure is indicated by an increase, and a fall by a decrease in the amplitude of oscillation.

[2] There seems to be considerable variation, in different subjects, of the optimum pressure for securing cardio-respiratory waves, particularly of the interference type. As the experiments proceeded somewhat lower pressures were used with better results. Figures 4 and 5 were taken in the neighborhood of diastolic pressure. All other records were taken much nearer the systolic than the diastolic level. Snyder (3) has shown that there is in most cases a reversal of the simple cardio-respiratory waves when the pressure in the cuff falls below the [illegible] level. It is probable that with the pressure maintained in this region [illegible] waves would suffer a similar reversal.

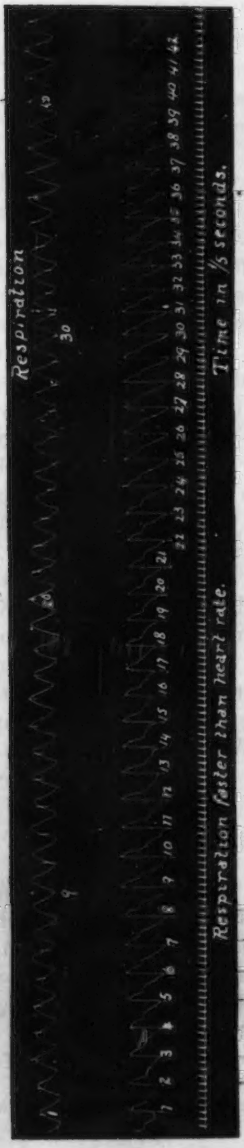
Respiration
Respiration faster than heart rate.
Time in ⅕ seconds.

Fig. 4

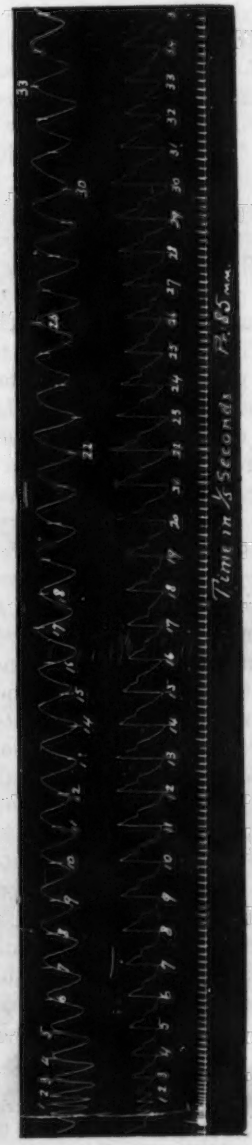
Time in ⅕ seconds P. 85 mm.

Fig. 5

In analyzing our records we have followed the common custom of laying off the heart beats on the respiratory tracing without making allowance for the time of transmission of the pulse to the cuff.

EXPERIMENTAL: *Simple cardio-respiratory waves of blood pressure.* All our records of blood pressure waves of this type (fig. 1) show, in the main, a rise of pressure during expiration, and a fall during inspiration (see fig. 17). This is in agreement with the findings of Erlanger and Festerling (2), but Snyder (3), in a record of twenty-eight cases, reports twenty as showing chiefly inspiratory rise and expiratory fall, and eight as showing chiefly expiratory rise and inspiratory fall of pressure.[3] In most of our experiments the pressure begins to rise toward the end of inspiration and to fall before expiration is completed (fig. 17). It is possible to get a complete reversal of the rise and fall in pressure by varying the depth and rapidity of breathing. We are in hearty agreement with Lewis (4, p. 254) when he says: "As respiratory curves of blood pressure are of very complex origin, and as the different factors involved in their production vary widely, it is not possible, either in man or animals, to state what the blood-pressure response to a particular respiratory act will be, unless the conditions and nature of the act are known."

Cardio-respiratory interference waves of blood pressure: When the rate of respiration is slightly faster or slightly slower than the heart rate we may conceive of cardio-respiratory cycles, each cycle comprising several heart beats and respirations, in which the number of respirations is greater by one, or less by one, than the number of heart beats. A cycle is completed when two beats (the first and last) fall at approximately the same time in respiration. When the heart rate is slower than the respiratory rate the pulsations of the heart fall progressively later in each respiration as they succeed each other. We shall henceforth speak of them as "advancing" along respiration. When the heart rate is faster than the respiratory rate the beats fall progressively earlier in each succeeding respiration, and we shall henceforth speak of them, under these circumstances, as "retreating" along respiration.

For the changes in blood pressure that occur in cardio-respiratory cycles we have already proposed the name of cardio-respiratory interference waves. It is convenient to think of each wave as beginning and ending in the pulsation of least amplitude. The length of the interference waves of blood pressure depends wholly upon how fast the

[3] For further discussion and bibliography on this subject refer to de Jager (5), Lewis (4), Erlanger and Festerling (2) and Wiggers (6), (7).

pulsations of the heart advance or retreat along respiration, it being evident that the faster the advance or retreat, the quicker the heart beats will complete a cycle, and the shorter the wave will be (see record in

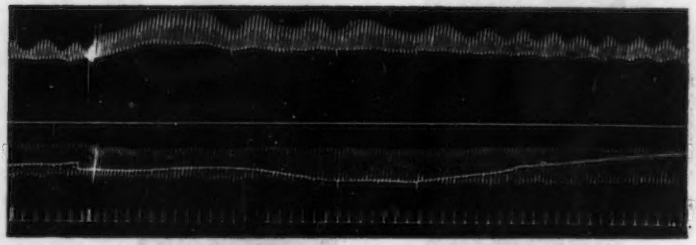

Fig. 6. Cardio-respiratory interference waves of the cat recorded with the Hürthle manometer connected with the carotid artery, showing a changing length of waves with a changing relative cardiac and respiratory rate. The upper tracing is the blood pressure, the middle is the respiration, and the lower the time record in seconds.

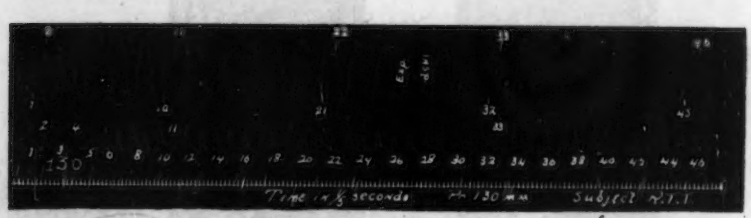

Fig. 7

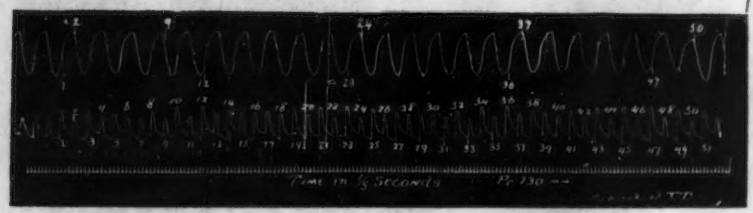

Fig. 8

fig. 6 which was taken from the cat, the blood pressure being recorded with the Hürthle manometer).

Cardio-respiratory interference waves may now be analyzed further according to whether they are produced by: *a*, respiration faster than

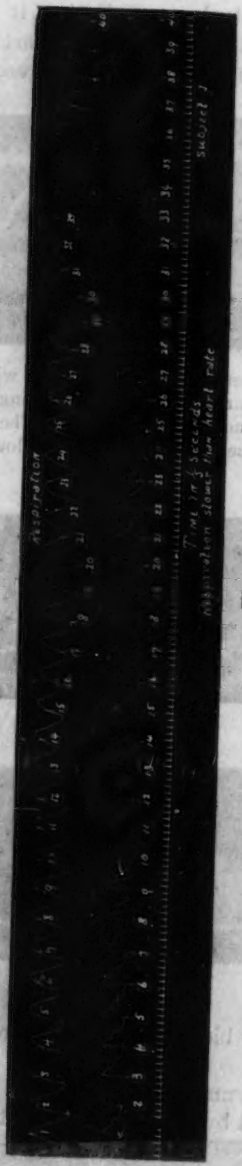
Respiration
Time in 5 seconds
Subject J
Respiration slower than Heart rate

Fig. 9


Time in 5 seconds

Fig. 10

the heart rate; *b*, respiration slower than the heart rate. For the sake of convenience, diagrammatic figures are used in the descriptions.

Figure 11a is a diagrammatic representation of a cardio-respiratory interference wave of blood pressure where the number of respirations is one more than the number of heart beats. The heart beats are laid off on the respiratory curve and numbered. It is apparent that the first four beats fall upon the inspiratory phases of respiration, while the remainder fall upon the expiratory phases. The relations of the blood pressure changes to respiration shown in figure 11a may be schematized in a simple form, shown in figure 11b, to make them more

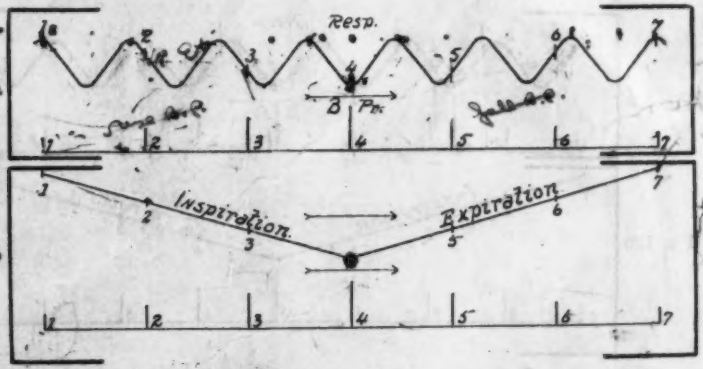

Fig. 11a is a diagram of a cardio-respiratory cycle, in which the respiration is faster than the heart rate. The pulsations advance along respiration, and are laid off on the respiratory curve. In figure 11b we have assembled all the beats on a single respiration in the same relative positions that they occupy in figure 11a. Note that the beats read from left to right, which is the direction of their movement along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively.

comparable to the changes occurring in the simple cardio-respiratory waves. Here we have taken a single respiration, represented in the usual way (the downstroke being inspiration and the upstroke expiration), and have transferred to it all the heart beats in the cycle, placing them in their proper positions in inspiration and expiration. Since the heart rate is slower than the respiratory rate, the pulsations of the heart advance in respiration in the schema just as the pulsations advance in respiration in the simple cardio-respiratory waves. Both respiration and blood pressure tracings are read from left to right. The relations thus schematized may be interpreted in two ways: *a*, taking into considera-

tion the direction of the movement of the heart beats relative to the respiration we may say that, as the pulsations advance along inspiration there is a rise in blood pressure, as they advance along expiration there is a fall in blood pressure; *b*, in the second interpretation we omit entirely all consideration of relative motion, and consider only the position of the heart beats in the respiratory phases. Note that the beats of least amplitude fall at the beginning of inspiration, while the beats of greatest amplitude fall at the end of inspiration.

Figure 12a is a diagrammatic representation of a cardio-respiratory interference wave of blood pressure where the number of respirations

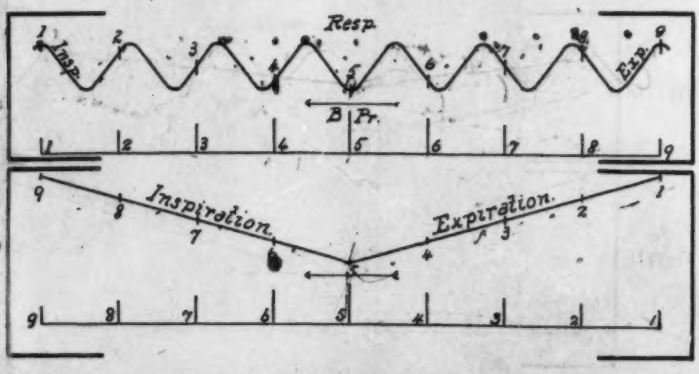

Fig. 12a

Fig. 12b

Fig. 12a is a diagram of a cardio-respiratory cycle, in which the respiration is slower than the heart rate. The pulsations retreat along respiration, and are laid off on the respiratory curve. In figure 12b all the beats are assembled on a single respiration in the same relative positions in which they fall in figure 12a. Note that the beats now read from right to left, which is the direction of their movement along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively.

is one less than the number of heart beats. The cardio-respiratory relations of figure 12a are schematically represented in figure 12b. Here again we have transferred to a single respiration all the heart beats in the cycle, placing them in their proper position in inspiration and expiration. The first five beats fall upon expiratory phases of respiration, while the last four fall upon inspiratory phases. Since the pulsations of the heart are retreating along, or lagging behind respiration (no. 1 falls at the end and no. 5 falls at the beginning of expiration), the blood pressure tracing in figure 12b must be read from right to left. The respiratory schema must, however, be read in the usual

way. The cardio-respiratory relations of figure 12b may likewise be interpreted in two ways: *a*, taking into consideration the direction of the movement of the heart beats relative to respiration we may say that, as the pulsations retreat along expiration there is a rise in blood pressure (it should be pointed out, however, that such a sequence of respiration and heart beat cannot possibly obtain in the simple cardio-respiratory wave); *b*, in the second interpretation disregarding entirely consideration of relative motion, and considering only the relative positions of the heart beat and respiration, we note that the beats of least amplitude again fall at the beginning of inspiration, while the beats of greatest amplitude fall at the end of inspiration. The waves differ only when the direction of the movement of the heart beat on the respiration is considered.

In figure 17 we have summarized graphically some of the data obtained from thirty-six subjects. The interference waves are plotted with reference to respiration according to the manner already described. The simple waves are plotted as they occur in the records. Each wave must be referred to the respiratory curve at the top of the graph. In no case is the amplitude of oscillation recorded. We have contented ourselves with recording the points of lowest and highest pressures, the lines connecting these points merely show that the pressure rises or falls as the case may be in the interval. It is evident that we have plotted a single wave only of each type. We have, however, analyzed many others, and have found almost invariably the same cardio-respiratory relations. We have in our records a few instances of simple and interference waves in which the cardio-respiratory relations are the reverse of what we have depicted, but these were always the exceptions even in the records in which they occurred. The numbers indicate the length of the individual waves as well as the number of beats making up the rise or the fall in pressure. Observe that the pressure changes of the interference waves follow more closely the changes of respiratory phase than do the simple waves.

Some very interesting effects may be obtained by having the subject breathe slightly slower, or slightly faster than half the heart rate. Under these circumstances we may get two sets of cardio-respiratory waves in progress simultaneously. One set is composed of the even-numbered, and the other of the odd-numbered beats. When one wave of blood pressure is at its crest the other is at its trough.

Figure 13a is a diagrammatic representation of a record such as figure 7, in which respiration is slightly quicker than one-half the heart

rate. The most obvious characteristic of such a record is an apparent alternating beat. Further analysis, however, shows that there are two sets of interference waves in progress at the same time, one being composed of the even-numbered, and the other of the odd-numbered beats. Figure 13b shows these waves plotted separately in their proper

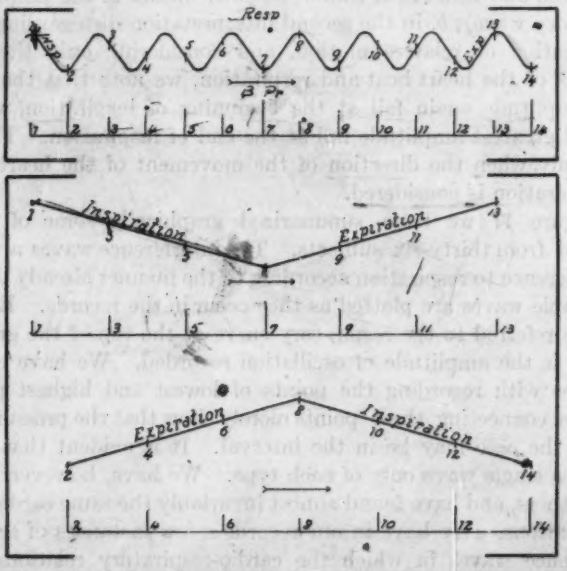

Fig. 13a

Fig. 13b

Fig. 13a is a diagram of double cardio-respiratory interference waves. The respiratory rate is slightly faster than half the heart rate. The pulsations advance along respiration, and are laid off on the respiratory curve. In figure 13b the odd and even-numbered beats respectively are assembled on single respirations in the same relative positions that they occupy in figure 13a. Note that the beats read from left to right, which is the direction of their movement along respiration. The points of highest and lowest pressures correspond to the beginning and end of inspiration respectively. Each of the double interference waves is identical with the type shown in figure 11b.

relation to respiration. Comparison of the two waves shows that they are of the same type, and that they bear identical relations to respiration. They are furthermore identical with the type of interference wave shown in figure 11b.

Figure 14a is a diagrammatic representation of a record such as figure 8, in which respiration is slightly slower than one-half the heart rate.

The double interference waves thus obtained are separated and shown schematically in figure 14b in their proper relation to respiration. Compare these waves with each other and with the interference wave represented in figure 12b. They will be seen to have identical relations.

The cardio-respiratory interference waves, whether of the usual (single) or alternating beat (double) types, have upon analysis shown

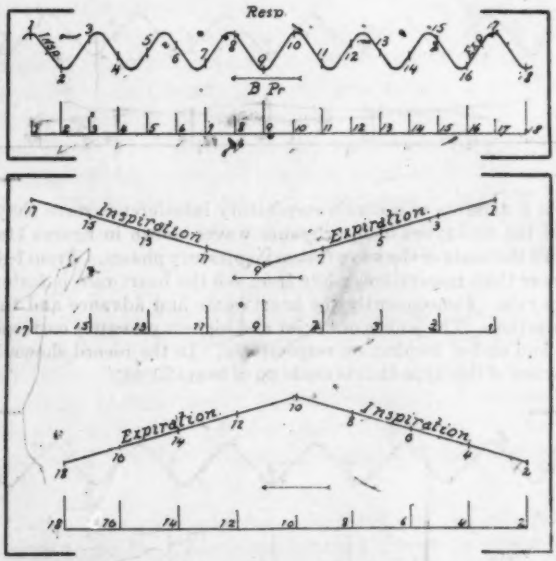

Fig. 14a is a diagram of double cardio-respiratory interference waves. The respiratory rate is slightly slower than half the heart rate. The pulsations retreat along respiration, and are laid off on the respiratory curve. In figure 14b the odd and even-numbered beats respectively are assembled on single respirations in the same relative positions that they occupy in figure 14a. Note that the beats now read from right to left, which is the direction of their movement along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively. Each of the double interference waves is identical with the type shown in figure 12b.

a relation to both phases of respiration, but records have been obtained which show that waves of blood pressure may be obtained that are related to either inspiration or to expiration alone.

Figure 15 is a diagrammatic representation of a wave of blood pressure that is related to inspiration only. From the beginning of inspiration

the beats advance till they reach the end of inspiration. At this point there is a change in the heart rate which causes them to retreat along the same respiratory phase. Figure 16 shows a wave related only to expiration. There is first a retreat and then an advance along this phase. These waves of blood pressure are equivalent to true inter-

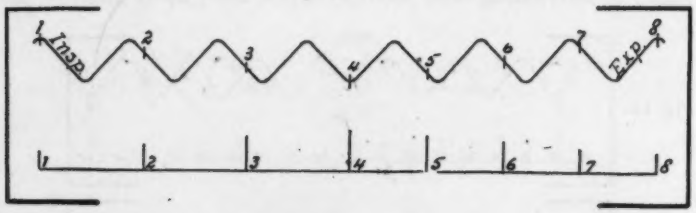

Fig. 15 is a diagram of a cardio-respiratory interference wave that is a combination of the two types of interference waves shown in figures 11a and 12a. Note that all the beats of the wave fall on inspiratory phases. From 1–4 the heart rate is slower than respiration, while from 4–8 the heart rate is faster than the respiratory rate. Consequently the heart beats first advance and then retreat along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively. In the record shown in figure 4 there is a wave of this type that is made up of beats 20–40.

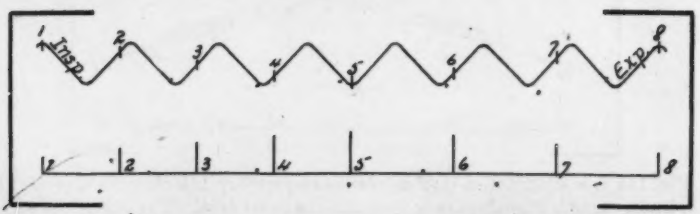

Fig. 16 is a diagram of a cardio-respiratory wave that is a combination of the two types of interference waves shown in figures 11a and 12a, but in this case all the beats of the wave fall on expiratory phases. From 1–5 the heart rate is faster than respiration, while from 5–8 the heart rate is slower than the respiratory rate. Consequently the heart beats first retreat and then advance along expiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively.

ference waves in spite of the fact that in the cardio-respiratory cycle the number of respirations is equal to the number of heart beats. Each half of a wave of this type has a relation to respiration that conforms to the conditions under which interference waves were found to occur. The changes in the heart rate that give rise to these waves are obviously

opportune, yet we have noticed several cases in our records where they occur.[4]

Up to this point we have dealt only with a shifting time relation between the pulse and the respiratory rates associated with changes in blood pressure, which appear to be related to respiration. Figures 9 and 10 show what occurs if the respiratory rate equals exactly *a*, the heart rate, or, *b*, one-half the heart rate. These conditions, difficult to obtain deliberately, are sometimes realized fortuitously. In figure 9 the heart and respiratory rates are very nearly the same from pulsations 5 to 12, and consequently the heart beats fall in approximately the same relative positions on respiration. As long as this relation is maintained there is no change in the amplitude of the pulse beats. Beyond pulsation 12, however, each successive beat falls earlier in respiration. The effect on the blood pressure is immediate; cardio-respiratory interference waves begin to form. In figure 10 the respiratory rate is for the greater part exactly equal to one-half the heart rate. Here we see the same phenomenon illustrated, namely, that beats which fall in the same relative position on different respirations do not change in amplitude.[5] Again we find that the beats of least amplitude fall roughly at the beginning of inspiration, while those of greatest amplitude fall roughly at the beginning of expiration.

Records such as figures 7, 8 and 10, occurring in the normal individual, suggest a possible new explanation for some cases of pulsus alternans.[6]

[4] It is obvious that a suitable change in the respiratory rate at the critical moment would be as effective in producing these waves as a change in the heart rate. Since respiration is under voluntary control, one should be able to secure them at will from a good subject.

[5] Figure 10 may be interpreted in other ways. This record may be considered as an example of the shortest possible interference waves. The cardio-respiratory cycle being made up of one respiration to two heart beats conforms to the conditions under which we have found these waves to occur (there is an excess of one heart beat over the number of respirations in the cycle). On the other hand, since the blood pressure changes are complete in the period of a single respiration, we might equally well call figure 10 a record of simple cardio-respiratory waves. Evidently the simple and interference types of waves are merged into one.

[6] As this paper is completed for the press our attention is called to a review (Journ. Amer. Med. Assoc., Oct. 22, 1921) of a paper by Aguilar on "Respiratory False Pulsus Alternans," occurring in a man suffering from bradycardia of nodal origin. It would be equally interesting to determine whether or not pulsus alternans of respiratory origin occurs in individuals suffering from dyspnea associated with rapid respiration.

Results on the dog and cat. The infrequency of distinct cardio-respiratory interference waves in the dog and cat, occurring as they do by accident, hardly warranted a series of experiments for their study alone. We have, however, collected some data in the course of other experiments which supplement the results on man. In these experiments (two on the dog and one on the cat) respiration was recorded as in man: blood pressure in the dog with the mercury manometer, in the cat with the Hürthle manometer. Cardio-respiratory interference waves with the respiratory rate approximately equal to the heart rate, and also to one-half the heart rate, were obtained. During the course of an interference wave in the dog, the mean blood pressure was highest when the pulse occurred at the end of inspiration, and lowest when the pulse occurred at the beginning of inspiration. In the cat both systolic and diastolic pressures showed the same relations to respiration; systolic pressure, however, showed the greatest fluctuations. Using pulse pressure as an index, cardiac output was greatest at the end of inspiration. In one experiment, in which simple cardio-respiratory waves were obtained as well as interference waves, the changes in blood pressure were similar to those reported in man. Therefore, the data obtained in the three experiments on the dog and cat are in agreement with the results reported above.

DISCUSSION

If figures 2, 3, 4, 5, 6, 7 and 8 are compared it will be evident that all the records show in the main the same thing, namely, that the pul-

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Fig. 17 is a graphic summary of a single blood pressure wave of each type obtained from the different subjects, the data being complete for thirty-two individuals.

Column 1 summarizes the data on simple cardio-respiratory waves of blood pressure.

Column 2 shows the cardio-respiratory relations of the interference waves where the respirations are in excess over the heart beats by one, that is, respiration is faster than the heart rate.

Column 3 shows the respiratory relations of interference waves where the heart beats are in excess over the respirations by one, that is, respiration is slower than the heart rate.

Corrections are marked for the onset of auricular and ventricular systole, indicating the distance each wave of blood pressure must be shifted to the left to show the relation of auricular and ventricular systole to the respiratory cycle. These corrections are obviously only approximately correct, and apply only to the waves of the interference type (columns 2 and 3). The corrections for auricular and ventricular systole in the simple waves are negligible owing to the greater average duration of each respiration.

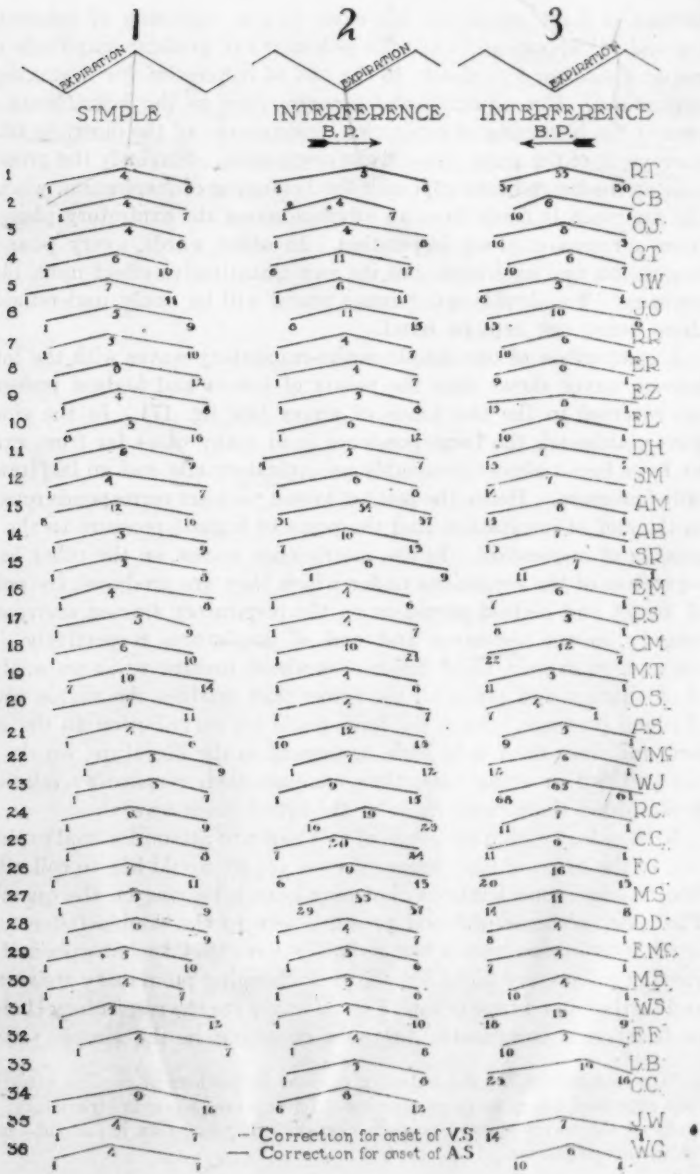
1
2
3
EXPIRATION
SIMPLE
INTERFERENCE
INTERFERENCE
B. P.
B. P.
RT
CB
OJ
CT
JW
JO
RR
ER
EZ
EL
DH
SM
AM
AB
SR
EM
PS
CM
MT
OS
AS
VM
WJ
RC
CC
FC
MS
DD
EM
MS
WS
FF
LB
CC
JW
WG
— Correction for onset of V.S
— Correction for onset of A.S

Fig. 17

sations of least amplitude fall close to the beginning of inspiration (or end of expiration), while the pulsations of greatest amplitude correspond more or less closely to the end of inspiration (or beginning of expiration). Consequently the pressure rises as the heart beats approach the beginning of expiration, irrespective of the direction of the movement of the pulse beats along respiration. Similarly the pressure falls as the heart beats approach the beginning of inspiration, whether the approach is made from an advance along the expiratory phase or from a recession along inspiration. In other words, every phase of inspiration and expiration has its own quantitative effect upon blood pressure. The double interference waves will be easily understood if these points are kept in mind.

A comparison of our simple cardio-respiratory waves with the interference waves shows that the points of lowest and highest pressures are reversed in the two kinds of waves (see fig. 17). In the simple waves, although the correspondence is in many cases far from exact, we have found almost invariably an expiratory rise and an inspiratory fall of pressure. Hence the point of lowest pressure corresponds roughly to the end of inspiration, and the point of highest pressure to the beginning of inspiration. In the interference waves, on the other hand, regardless of the conditions under which they are produced, the points of lowest and highest pressures on the respiratory tracing correspond roughly to the beginning and end of inspiration respectively. An attempt to explain these differences would involve us in an analysis of the nature and action of the forces that produce the simple waves of blood pressure. Since we have made no contribution to the subject, and since there is so little agreement in the literature, we do not feel justified in doing more than compare their respiratory relations, as we found them, with those of the interference waves.

Neither is it within the scope of this paper to attempt a final explanation of the cause of interference waves, yet we would like to call attention to some considerations that may have a bearing on the question. The quick changes of blood pressure seen in the double (alternating beat) interference waves are probably too rapid to be explained by varying pulmonary blood capacity, or changing pulmonary resistance; and further, in no type of interference waves are the respiratory changes in heart rate comparable to those occurring in the simple waves.[7]

[7] One subject (S.M.) did indeed give clear indications of cardiac variations that appeared referable to respiration. These were, however, transitory, and many of the waves showed no such changes. The question is one that needs further investigation.

For these reasons we believe the production of interference waves to be due primarily to rapid changes in the movement of blood in and about the base of the heart, resulting from changing intra-thoracic pressure.[8]

SUMMARY

The effects of rapid breathing were compared with those of more normal breathing upon the systolic blood pressure in man. Supplementary data were also obtained on the dog and cat.

For the well-known changes of blood pressure that occur during a single respiration, and which are more or less synchronous with the changing respiratory phases, we have proposed the name of *simple cardio-respiratory waves* to distinguish them from those waves produced by rapid breathing.

The oscillations of pressure elicited during rapid breathing by the interference method we have designated as *cardio-respiratory interference waves.*

The most striking difference in the respiratory relations of the simple and interference waves is that in the simple waves the blood pressure changes are complete within the period of a *single* respiration, while in the interference waves the gamut of the blood pressure changes is run through in the interval of *several* respirations.

[8] We have already called attention to the fact that in analyzing our records we have made no correction for the time necessary for the transmission of the pulse to the cuff. Neither have we made correction for the beginning of ventricular or auricular systole, but have determined the position of the heart beats with reference to the appearance of the pulse at the cuff to make the results comparable with those reported in simple cardio-respiratory waves. These results are summarized in figure 17. We have added two corrections to this figure, showing the moment of onset of ventricular and auricular systole in the respiratory cycle corresponding to the pressures which are plotted. For example, to determine the relation of the onset of ventricular systole associated with the highest pressure to respiration, the blood pressure wave should be shifted to the left, the designated distance equivalent to 0.06 second. To determine the same relation for the onset of auricular systole, the curve should be shifted in the same direction, the designated distance corresponding to 0.16 second. In making these corrections the points of high and low pressures will now be found to anticipate the respiratory changes of phase. If we accept the common view that an increase in the negative pressure of the thorax favors venous filling by its aspiratory action, we might expect the points of highest pressure to correspond with the onset of auricular systole and the end of inspiration. But we cannot say with certainty how changing intra-thoracic pressure acts. The difference in thickness of auricle and the left ventricle might, for example, hinder the flow of blood from the auricles to the ventricles during auricular diastole when inspi-

The production of interference waves of blood pressure is dependent upon the establishment of cardio-respiratory cycles, in which the number of respirations is greater by one or less by one than the number of heart beats making up the waves and occurring in the same time interval.

When these conditions are fulfilled we may conceive of the heart beats as moving through respiration, the direction of the movement being determined by the relative rates of the heart and respiration; that is, whether the respiratory rate is slower or faster than the heart rate. A cardio-respiratory cycle is complete when two beats (the first and last of the interference wave) fall at approximately the same time in respiration.

We have found that, whereas in the cardio-respiratory interference waves the highest and lowest pressures were associated approximately with the beginning of expiration and of inspiration respectively, in the simple respiratory waves these relations were reversed; that is, the points of highest and lowest pressures correspond roughly to the beginning of inspiration and of expiration respectively.

Without definitely assigning the responsibility for the production of interference waves to any particular respiratory factor, we are inclined to favor the hypothesis that they are primarily due to the changing intra-thoracic pressure accompanying respiration.

It is possible, by breathing slightly slower or slightly faster than half the heart rate, to produce double interference waves of blood pressure; that is, under these conditions, two waves of blood pressure may be in progress simultaneously. Each of the double waves is formed by alternate heart beats, one being made up of the even-numbered and the other of the odd-numbered beats. Double cardio-respiratory interference waves are to be explained in the same manner as the single waves.

Supplementary data obtained from experiments on the dog and cat are in agreement with those obtained in man.

Cardio-respiratory interference waves particularly of the double type with alternating beats occur spontaneously in sacrifice experiments in dogs and cats. We, therefore, point to our work as occasionally explaining pulsus alternans and blood pressure waves of the third order occurring in man.

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STUDIES ON ALKALIGENESIS IN TISSUES

I. Ammonia Production in the Nerve Fiber during Excitation[1]

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Immediately after the publication of an article (1) in which the author reported that the resting nerve respires and that this respiration increases during the passage of the impulse, he made some attempts to devise a quick and easy method practicable for ordinary class experiments to demonstrate this increased metabolism during the stimulation. On account of the necessity of an elaborate device to make air free from CO_2, the principle used in his original apparatus could not be used. Since we know today more about the proper use of indicators than in early days, when physiologists used them for detection of CO_2 in metabolism experiments on nerves without any degree of success, and since we know the exact amounts of CO_2 produced in the nerve under various conditions, the indicator method was next tried. We immersed the nerve in Ringer's solution, saturated with phenolphthalein and containing enough alkali to give a slight pink color but not enough to affect appreciably nervous activity. Although this method was good enough to show that the resting nerve gives off an acid (CO_2), yet it would not show a decided difference in rates of decolorization in tubes containing resting and stimulated nerves.

This failure of detection of an increased carbon dioxide by the stimulated nerve meant one of two things. Either the $Ba(OH)_2$ method the author previously used was wrong and the stimulated nerve does not give any more CO_2 than the resting, or the increased CO_2 production is masked when an indicator method was used. Because of several hundred repetitions with the same results of his original method, demonstrated to many and confirmed by all of his students who learned the proper use of his apparatus, this apparent failure with the indicator

[1] Preliminary report of this work was given before the Chicago Meeting of the American Physiological Society, 1920, and its abstract appeared in this Journal, 1921, lv, 282.

method was not taken by him as conclusive evidence against his original contention but was considered to be due to some difficulty with the indicator method.

With an assumption, therefore, that his original method of estimating CO_2 is accurate and that CO_2 production does increase in the nerve during excitation, we proceeded to inquire what could be the interfering factors if the indicator method was used.

There are two obvious conditions which might mask the increase of acidity due to production of more CO_2—one is the simultaneous production of any base-forming compounds; the other is the presence of buffers in the tissue itself or its production in or diffusion out of the tissue.

During preliminary experiments, the author discovered that when two nerves of equal weight were placed in chamber A and B of his CO_2 apparatus and hemispheres of Nessler's solution were introduced instead of $Ba(OH)_2$, the Nessler drop in the chamber in which the stimulated nerve is placed gave a brownish precipitate much quicker than that of the resting nerve. This suggested the possibility of more NH_3 formation in the stimulated nerve, but it was not necessarily NH_3 since CO_2 is known to give a precipitate with Nessler. To scrutinize this difficulty, drops of NaOH solution were placed in both chambers for the purpose of eliminating any CO_2 which we knew to be produced. The same result could be obtained as before, showing that the precipitate was not due to CO_2, but it was impossible to use this method for estimating quantities. The first difficulty was in producing a perfectly clear Nessler drop which is the essential requirement for quantitative determinations in the biometer. The second difficulty was that the stopcock caused a great deal of trouble on account of its strong alkali. The substitution of a pinchcock for the stopcock caused a further difficulty in that it was impossible to place a stationary hemispherical drop of Nessler's reagent on the top of the tube.

In spite of these difficulties and the lack of quantitative data, however, the conclusion was warranted that there is at least one other volatile compound produced besides CO_2. Consequently the method of immersing an isolated nerve directly into a solution to estimate CO_2 by measuring the rate of change of the hydrogen ion concentration might cause a very serious error, unles we can ascertain that not only the other gas has no influence upon H^+ produced by CO_2, but also that there are no other compounds diffusing in or out of the tissue in the solution which effects the reaction of the medium.

In view of this experience and considering the easy availability of standard indicator tubes now on the market for determination of the H^+ concentration, the author expected some one to report similar results, namely, that the increased metabolism accompanying stimulation cannot be detected by a direct indicator method. It is not surprising, therefore, to see an article (2) by A. R. Moore of Rutgers College in which such a negative result is reported; and to him all the credit for this rediscovery of the negative result obtained by very early workers should be given. It is, however, surprising indeed to see him dismiss this problem by saying that "*The nerve impulse does not depend upon processes leading to the production of carbon dioxide.*"

The present communication is, however, not to show whether or not an indicator method should be used for estimation of CO_2 in the nerve fiber, nor to consider all factors which are sufficient to explain why contradictory results were obtained with the indicator and our original CO_2 methods (3). It is, first, to prove that the nerve fiber gives off a basic substance simultaneously with an increased production of carbon dioxide and that this substance is most probably ammonia; and second, to consider the relationship between irritability and ammonia formation in the nerve under various conditions.

EXPERIMENTAL: *Part 1. Preliminary consideration.* If the sciatic nerve of a frog is placed in Ringer's solution, made slightly alkaline and colored with any weak acid indicator like phenolphthalein, sooner or later the fluid gives an acid reaction. If, therefore, we assume that the failure to detect the increase of CO_2 during the stimulation is due entirely to ammonia alone, we should expect that the maximum amount of ammonia production cannot be greater than that necessary to neutralize 8.7×10^{-7} grams of CO_2 for 10 mgm. of the nerve during 10 minutes of respiration using phenolphthalein as an indicator, since for the same units, a resting nerve gives off 5.5×10^{-7} grams and an activated nerve 14.2×10^{-7} grams CO_2 at ordinary room temperture, provided of course our previous estimation of CO_2 is correct.

Theoretically, 34 grams of NH_3 should neutralize 44 grams of CO_2, and 8.7×10^{-7} grams $\times \frac{34}{44} = 6.7 \times 10^{-7}$ grams of NH_3 should be required to completely neutralize just the amount of increased CO_2 produced during excitation. However, since NH_4OH is more highly ionized than H_2CO_3, the probability is that far less an amount of NH_3 than 6.7×10^{-7} grams will be able to maintain a definite level of H^+ concentration in presence of 8.7×10^{-7} grams of CO_2, which is the

amount increased in 10 minutes by 10 mgm. of the stimulated sciatic nerve of a frog, under ordinary conditions.

How far this hydrolysis of $(NH_4)_2CO_3$ will affect the turning point of the indicator will depend not only on the concentration of $(NH_4)_2CO_3$, and kind of indicators and the amount of free CO_2 present, but also upon the presence of other salts as well as on temperature. Since available data on these points are almost inapplicable to the condition under which we are working, the exact relationship between NH_3 and CO_2 in respect to maintenance of certain H^+ concentration in dilution approximately that of our problem is under separate investigation, the results of which will be published elsewhere in conjunction with Mr. L. S. Friedman.

The point of the foregoing consideration is, however, to show that the amount of NH_3 produced under the conditions stated might be far less than 6.7×10^{-7} grams and that any method for detection of the gas must necessarily be exceedingly delicate.

Part 2. Qualitative experiments. In the following experiments we shall see whether or not the living nerve gives off something else besides CO_2, and if so, we shall attempt to identify the nature of this compound. In these cases, we used more than 100 mgm. of fresh sciatic nerves of the frog, and let them respire more than 15 minutes.

a. Does the resting nerve give off something else than CO_2 when immersed in Ringer's solution?

Experiment 1. Tube i: 3 cc. Ringer + nerves; tube ii: 3 cc. Ringer.

When tubes i and ii are Nesslerized after a definite time of respiration, tube i will show a more intense yellowish color than ii. This suggests a possibility of NH_3 formation but not necessarily, since it is known that CO_2 and other compounds by virtues of forming precipitates or similar colors, might produce different colorations.

The following experiments may eliminate CO_2 factor.

Experiment 2. Tube i: 3 cc. Ringer + nerve; ii: 3 cc. Ringer.

At the end of respiration, 1 cc. of N/800 H_2SO_4 is added to each tube and placed in boiling water for 5 minutes. The obvious reason of this treatment is of course to boil off CO_2 without losing NH_3 if present.

Since under this condition tube i is still more intensely colored after Nesslerization than the control, it is certain that the living nerve when immersed in Ringer's solution, gives off something besides CO_2.

b. Is it ammonia? The experiments cited above do not rule out the possibility that the slight amount of neutral ammonium salt might have diffused out of the nerve or that some compound other than NH_3 might be diffused or produced from it.

1. If it is ammonia; it should be volatile; we ought to be able to absorb it out of the air by acid.

Experiment 3. Tube i: 1 cc. of N/800 H_2SO_4 + nerve (hanging without touching the solution); tube ii: 1 cc. of N/800 H_2SO_4.

At the end of respiration, the tubes are shaken without moistening the nerve and the tissue removed, and both of the tubes are immersed in boiling water for 6 minutes. To the bottom of cooled solutions, 1 cc. of Graves' reagent[2] is carefully introduced. Graves' reagent gives a white precipitate with ammonia, Tube i gives a faint white cloud at the junction of the 2 fluids. Tube ii gives no precipitate. The ring test thus performed shows conclusively that the compound is volatile and gives an insoluble complex salt with $NaHgCl_3$ in alkaline solution, exactly in the same manner as do NH_4 salts.

2. If this is ammonia, it should not only be volatile, but also should form a base. The base-forming property of this compound can easliy be demonstrated by the following experiments.

Experiment 4. Tube i: 1 cc. Indicator[3] + unstimulated nerve; ii: 1 cc. Indicator.

The tubes are corked and allowed to stand.

At the end of respiration these tubes are shaken and the tissue is removed. The tubes are then immersed open in boiling water for 6 minutes. If at the end of the boiling there is not a detectable difference in the color, then to each tube add alternately drop by drop of distilled H_2O kept in *ordinary glass* to each tube. In the course of adding this exceedingly weak alkaline solution, it will be noted that tube *i* which has had the nerve will decolorize first, then *ii*. Since this indicator will lose its pink color when H^+ concentration reaches pH = 5 to 6, it is evident that as less alkali needs to be added to tube i this compound given off by the nerve has a base forming property.

c. Does the activated nerve give off this compound more than the resting? In similar experiments as described above but by substituting for the control the tube containing a stimulated nerve, it can be demonstrated that the nerve when stimulated gives off more of this compound than the resting.

d. Is this an amine or ammonia? 1. The fact that this compound is volatile and forms a yellow complex salt with Nessler's reagent and a white precipitate with $NaHgCl_3$ (Graves' reagent), strongly suggests that it is NH_3 gas, but does not absolutely rule out the possibility of

[2] See page 525.
[3] See page 528.

its being one of the volatile amines. There are theoretically many ways by which we may be able to differentiate amines from NH_3, yet we have not succeeded in applying them satisfactorily to such a small concentration as that with which we are dealing. If, however, the quantitative data obtained by two methods based on entirely different chemical properties of the substance, agree within experimental errors, then the identity of this compound can easily be ascertained.

Thus by estimating basicity produced by this compound, we may calculate it on the basis of NH_3, and compare the results with those obtained by Nessler or Graves' methods using standard ammonium salt. If such results do not agree with each other, we may calculate the basicity on the basis of all volatile amines, and compare the results obtained from the other method using standard solution of various alkyl ammonium salts.

Considering the difficulty and large sources of error obtained by turbidity experiments with exceedingly minute amount of this substance, the results obtained by these two methods are satisfactorily concordant to show that it is ammonia. (See page 523.)

On the basis of this evidence, we shall from now on call this compound ammonia until we shall have evidence to the contrary.

Part 3. Quantitative methods.[4] The two well-known properties of NH_3, used in ordinary methods, can be applied for measurement of exceedingly minute quantities. With slight modification and a great deal of care, one can detect an amount of NH_3 gas as small as 0.000,000,1 gram either by means of converting it to a complex salt with Hg. (Nessler or Graves'), or by measuring the amount of base formed by the gas. Since, however, the presence of CO_2 will interfere with all the methods based on these properties (the basicity in the greatest degree), it is absolutely necessary to have a device to eliminate CO_2. It is equally essential to avoid a direct contact between the tissue and liquid in which NH_3 is to be estimated, on account of possible diffusion of neutral NH_4 salts or other nitrogenous extractives that would react with direct Nesslerization or Graves' reagents, and on account of the possible presence of buffers either diffused out of the tissue or present in the tissue itself that would mask the true basicity attributed to ammonia alone. The amount of NH_3 gas actually given off by the nerve can only be estimated by suspending the tissue over a very small amount of dilute acid, the concentration of which should be kept con-

[4] For checking up the method as well as certain experiments, the author is greatly indebted to Miss Olive Pearl Lee and Dr. H. Sugata.

stant not only for all the nerves and controls, but also for the standard in each set of experiments.

A. Ring test with Graves' reagent. A new precipitant for ammonia recommended by Graves[5] is just as sensitive as that of Nessler. When used as a ring test, moreover, we found that as small as 1×10^{-7} grams N in form of NH_3 can be easily detected. This formation of a ring depends, however, upon two factors, i.e., concentration of the acid and of NH_4. For example in N/400 H_2SO_4, 2.5×10^{-7} grams N per cc. is barely detectable, while in N/800 H_2SO_4, 1×10^{-7} grams N will form a white ring within 3 to 4 minutes.

By varying acidity, therefore, over which the nerve is to respire, and by determining how much NH_4 is necessary to form a ring in the acidity in which a positive ring was formed, and over which nerve was suspended, we can estimate how much NH_3 is produced from the given nerve during a given period of respiration.

There are two main difficulties with this method. First, it is exceedingly difficult to determine the absolute point at which ring formation occurs, the speed of which depends not only on the concentration of NH_3, acidity, but also temperature. Second, it involves maintenance of a series of standard acids whose blank ammonium contents must be kept constant. If this varies, there is no way of correcting an error without performing a series of elaborate quantitative estimations with the different ammonium standards in different concentrations of the acids. Although this latter difficulty can be eliminated to a great degree by making up for each experiment a series of standards of the ammonia in the same acidities which are used for absorption of NH_3 from the nerve, yet the amount of acidity reduced by the NH_3 has not been reckoned with the standard.

In addition to this, any quantitative method based on such a formation of a barely visible ring is impossible to make free from a personal factor. But for a quick qualitative-quantitative test to determine which gives more NH_3, this ring test with Graves' reagent is very convenient and reliable.

B. Nephelometer method. This is essentially Graves' method. Since, however, the amount of NH_3 is so small a minor modification is necessary. In this, the use of starch is omitted on account of the

[5] Graves' reagent is made up as follows: To 80 grams of NaCl, are added 130 cc. H_2O and 100 cc. of cold sat. $HgCl_2$ solution with shaking. When the salt is practically all dissolved, 70 cc. of sat. Li_2CO_3 are added slowly while shaking, so that no mercury oxide forms on the side of the flask.

fact that the precipitate is so little as to maintain almost permanent turbidity without the use of any protective colloid. In order to keep the turbidity at the possible maximum, the dilution with NH_3-free H_2O is omitted. Ordinarily, for each cubic centimeter of N/800 H_2SO_4 over which the nerve is suspended, 10 cc. of Graves' reagent are added and the resulting turbidity is matched against the standard which contains 10 cc. of the reagent and 1 cc. of the standard $(NH_4)_2SO_4$ made up in N/800 H_2SO_4.

Although this method should be theoretically far superior to that of the ring test, the accurate matching of this exceedingly small turbidity is exceedingly difficult with the best nephelometer on the market even to the best and most experienced person. Any effort to increase the amount of turbidity by using a larger number of the isolated nerves is apt to introduce very serious physiological errors.

In addition to these, the method is so tedious on account of necessity of having perfectly clean and dry tubes ready, that it is impossible to run the quantitative estimations more than two dozen a day without having two or three dozens of nephelometer tubes that are uniform in all regards. Expense has precluded obtaining any such number. Unless for the purpose of identifying NH_3 by checking the result with the titration method, we have discarded the use of this method for a routine determination of NH_3.

C. Titration method: 1. Principle. By far the most satisfactory method, we found, is a titration method. The method itself has no claim of originality. The nerve is suspended over a definite quantity of an acid which contains a proper indicator. At the end of the respiration, CO_2 is driven off by immersing in boiling water 6 minutes, and the remaining acidity is titrated drop by drop with an alkali.

In spite of simplicity of the principle, however, there are many necessary details and precautions, without which the method is not dependable.

2. Apparatus. No special apparatus is necessary except that all, unless otherwise stated, should be made of Pyrex glass. Respiration and titration are performed in ordinary 6 inch Pyrex test tubes. One of the greatest sources of error and difficulty will be with these test tubes. For a successful experiment, it is convenient to have over 100 of the Pyrex test tubes and have them ready according to the following procedures:

These tubes are boiled in about 10 per cent H_2SO_4 for one hour and washed thoroughly with ordinary distilled water several times. Finally

these are again rinsed at least twice in ordinary distilled water, filling the tube to the top in each rinsing. These are then boiled in ordinary distilled water for one hour twice, and finally boiled in NH_3-silicate free[6] H_2O for one hour.

After being heated in a hot oven for 2 hours, the tubes are directly transferred in a con. H_2SO_4 desiccator. The tubes thus prepared should be tested for their cleanness in the following way.

When a solution of phenolphthalein made alkaline so that it is just perceptibly pink is added to the tube, it should not be decolorized in the cold. The faint pink color of methylene blue-methyl red (see p. 528) should stay permanent in the test tube without heating. When 1 cc. of the same indicator-acid solution is heated in this tube for 6 minutes in boiling water, the pink color should not be intensified. On the other hand, if this color changes to deep yellow the tube should be discarded.

All the tubes which stood a successful test were rinsed with redistilled H_2O and boiled again in redistilled H_2O once, and finally boiled in water free from ammonia and silicate for one hour. After being heated in a hot oven for 2 hours, they are directly transferred in a con. H_2SO_4 desiccator, ready for the experiment.

The tubes once used successfully for ordinary respiratory experiment should be subjected to similar cleaning as described in the last paragraph, i.e., they need not be boiled again in the strong acid.

In spite of all these precautions, one often finds a tube which is contaminated with alkali or acid. In the course of the experiment, one may also desire to add an excess of alkali or acid for various reasons to a tube. Such tubes should be set aside, separate from the regular tubes, and be subjected to more careful washings than the tubes which are used for routine quantitative experiments.

3. Solutions: a. Water free from NH_3 and silicate. Ordinary method of preparation of NH_3-free-H_2O is employed in preparing this water, care being taken, however, to use Pyrex glass in every part of the distillation apparatus where water or its vapor comes in contact with it. Thus it is recommended to use Pyrex distilling flask, a condenser whose inside tube is made of Pyrex, and receptor and adapter, all made of the same material. This NH_3-free-H_2O freed as much as possible from silicate is kept in a Pyrex bottle or flask tightly stoppered with paraffined cork.

[6] See last paragraph, this page.

b. Indicators: 1. Methylene blue solution, 0.5 gram of methylene blue, special,[7] is dissolved in 200 cc. NH_3-silicate-free H_2O. A 0.025 per cent solution is made from this stock solution by diluting 10 cc. to 100 cc. with NH_3-free H_2O.

2. Methyl red. Methyl red, special, recrystallized from alcohol, is saturated in 50 per cent of redistilled alcohol at room temperature.

3. To make methylene blue-methyl red (MB-MR) indicator. For 1 cc. of 0.025 per cent of methylene blue, add 10 cc. of methyl red solution.

4. Ordinary alcoholic solution of phenolphthalein.

c. Standard solutions: 1. N/20 H_2SO_4 solution, made up from a standard acid by diluting with NH_3-free H_2O, is kept in several small bottles well protected from the air.

2. Standard alkaline solution. The error of the experiment will depend a great deal upon the concentration of the standard alkali solution. If it is too low, the end point will not be sharp. If it is too high, the NH_3 can not be detected. Any alkaline solution which contains available alkalinity of N/10,000 or about, is satisfactory, that is when 0.1 cc. N/20 H_2SO_4 + 1.2 cc. MR-MB is made up by this solution to 100 cc., the pink color should disappear when immersed in boiling water for 6 minutes. If such alkali solution is completely ionized, H^+ should correspond to between pH 8–9, just the turning point of phenolphthalein, and of course way up on the alkali side of MB-MR. When, however, such a solution contains a trace of silicates, even if it is decidedly acid to phenolphthalein, the solution will often be too strongly alkaline for our purpose. Therefore as long as we are not sure of absence of silicate in the water, the ideal method of preparation of this solution on the basis of free H^+ concentration alone as determined by indicators will not be safe unless it is checked by a titration in a manner described below.

The method by which one can prepare the satisfactory concentration of alkaline water is as follows. Several liters of NH_3-silicate-free H_2O is poured into a large Pyrex flask and a few drops of phenolphthalein solution added. After addition of each drop of N/20 NaOH, the

[7] Presence of proper amount of methylene blue is very important for detecting the end point. Since we all know that no two brands of methylene blue on the market are the same, we have specified the amount on the basis of methylene blue, special, prepared by Coleman and Bell, Norwood, Ohio. If one prefers to use other brands, it will be necessary for him to determine proper concentration of the indicator to use.

bottle is tightly stoppered with paraffined cork and shaken until a faint but distinct pink color persists. Then enough N/20 H_2SO_4 is added to barely decolorize the pink color. The flask is filled with a Pyrex syphon; the inlet of air is protected with 15 per cent NaOH and concentrated H_2SO_4. The tip of the syphon is also provided with cork to which a Pyrex test tube can be inserted to protect against diffusion of the gases through the tip.

This solution should be tested out for its proper alkalinity in the following way.

To a Pyrex flask marked at 100 cc., 0.1 cc. of N/20 H_2SO_4 and 1.2 cc. of MB-MR are added using Pyrex pipettes. The volume is made up to 100 cc. mark with the alkaline solution just described. If the resulting solution is greenish yellow, it is apt to be too strongly alkaline. It should be colored faintly pink in cold, but should become a pale greenish yellow when a few cubic centimeter of this are placed in the Pyrex test tubes, previously tested, and immersed in boiling water for 6 minutes.

When 0.2 cc. of N/20 H_2SO_4 is taken and tested in the same way, the color should be faintly pink after immersion in the water for the same period of time. In other words the concentration of the alkali should be somewhere around N/10,000.

3. Standardization of the standard alkali. This approximately right alkali solution is standardized as follows.

To each of four Pyrex flasks marked at 100 cc. various fractions of cubic centimeter from 0.5–0.2 cc. of N/20 H_2SO_4 accurately measured with Pyrex pipette and 1.2 cc. of MB-MR, measured also with Pyrex pipette, are added, and made up to the volume with the alkali solution. One cubic centimeter of each is measured off by Pyrex pipette from each flask into a clean Pyrex test tube, previously tested, and immersed in boiling H_2O. At the end of 6 minutes, each tube is titrated drop by drop with the same alkaline solution. The typical result of titration of a particular standard solution we made up as shown in table 1.

The average of these determinations, then, shows that 7.2 drops of alkali solution were necessary to neutralize 1 cc. of N/20,000 H_2SO_4, since each flask contains increment increase of 0.1 cc. of N/20 per 100 cc. and since we took for titration 1 cc. only. On the basis of this titration, each drop of the alkali is equivalent to 1/7.2 cc. of N/20,000 alkalinity, which in terms of NH_3 corresponds to 0.000,000,12 gram.

It should be noted that in the above calculation, we have not ignored the effect of the indicator itself, but have eliminated it on account of the

fact that each cubic centimeter of the acid we took contained exactly the same amount of indicator, and by subtracting number of drops from the one above, the acidity due to indicator itself is canceled.

TABLE 1

TUBE	CUBIC CENTIMETERS OF N/20 N_2SO_4	CUBIC CENTIMETERS OF MB-MR	MADE UP WITH THE ALKALINE SOLUTION	NUMBER OF DROPS REQUIRED TO NEUTRALIZE 1 CC. OF THE SOLUTION	DIFFERENCE FOR EACH TUBE
					drops
A	0.5	1.2	100	22	7.0
B	0.4	1.2	100	15	7.5
C	0.3	1.2	100	7	7.0
D	0.2	1.2	100	0	
Average drops.....					7.2

From the same data, however, we may calculate the acidity contributed by the indicator, as shown in the following table.

TABLE 2

TUBE	CUBIC CENTIMETERS OF N/20 H_2SO_4	CUBIC CENTIMETERS OF MB-MR USED	MADE UP WITH THE ALKALINE SOLUTION	NUMBER OF DROPS OF THE ALKALINE SOLUTION NECESSARY TO NEUTRALIZE 1 CC. OF THE SOLUTION	NUMBER OF DROPS CONTAINED IN 1 CC. PIPETTE USED TO MEASURE THE ACID-MB-MR	SUM OF DROPS	NUMBER OF DROPS REQUIRED TO NEUTRALIZE 1 CC. N/20000
A	0.5	1.2	100	22	18	40	40/5 = 8.0
B	0.4	1.2	100	15	18	32	32/4 = 8.0
C	0.3	1.2	100	7	18	35	25/3 = 8.3
D	0.2	1.2	100	0	18	18	18/2 = 9.0

Average for first three = 8.1 drop. Average for all, including the last which was already decolorized before titration = 8.3. Average of these two = 8.2; 8.2 − 7.2 = 1 drop = the amount of alkali required to neutralize 1.2/100 cc. of MB-MR indicator, the amount of indicator contained in 1 cc. of MB-MR-acid.

If we can ignore the acidity contributed by the indicator, the standardization of the alkali solution can be done by a simple method. For instance, take 0.5 cc. of N/20 H_2SO_4 and 1.2 cc. of MB-MR, and dilute it to 100 cc. with solution to be standardized. Titrate 1 cc. of this solution according to the method described above. Add the number of drops contained in 1 cc. of the pipette used for titration, to the

number of drops of the alkali required to neutralize the acid-MB-MR. The sum of the drops divided by 5 will be equivalent to 1 cc. of N/20,000. The general formula for this calculation is as follows:

$$\text{One drop} = \frac{1}{\frac{(a+b)}{c}} \times 0.000{,}000{,}85 \text{ gram } NH_3$$

where a = number of drops required to neutralize 1 cc. of the solution.

b = number of drops contained in each cubic centimeter of the pipette used for titration.

c = number of cubic centimeters of 1/200 cc. H_2SO_4 (number of tenths of cubic centimeter of N/20 H_2SO_4) used to make the solution and 0.000,000,85 gram is amount of NH_3 contained in 1 cc. of N/20,000 NH_4OH solution.

If one uses always the same fresh indicator, and is sure of the amount of acid it contributes, then the following formula can be used, since 1.2/100 cc. of MB-MR does neutralize 0.000,000,02 gram of NH_3.

$$\text{One drop} = \frac{1}{\frac{(a+b)}{c}} \times 0.000{,}000{,}85 \text{ gram } NH_3 + 0.000{,}000{,}02 \text{ gram.}$$

Under strictly ideal experimental conditions, there are 3 factors which will determine the error of determination: a, correct standardization of the alkali; b, any factors which influence size of the drop, and c, end point. The first two factors concern the accurate estimation of the amount of alkali taken. If one wishes, therefore, to be exceedingly accurate, instead of measuring it by drops, use of a very small Pyrex pipette, accurately calibrated to hundredths of a cubic centimeter will narrow the limit of error. The effect of temperature on the number of the drop should, of course, be always remembered.

Although the end point is sharp within ½ drop of the alkali we ordinarily use, the error will be enormously great if the number of total drops of the alkali used is very small. It is highly desirable for one to decide what point is the end and use that criterion for all the experiments, both standardizations of the alkali, and titration of the remaining acid after the respiration. The point of change from pink to faint greenish yellow is the most sharp. Many unnecessary errors will be eliminated if one uses a control tube in which the same amount of the indicator is placed and which has the greenish yellow color to be compared.

During titration, care should be taken not to let the alkali drop touch the side of the tube, but to let it drop directly into the solution.

4. Preparation of MB-MR-acid solution. If we know the exact concentration of the alkali, theoretically any strength of the acid should be satisfactory, provided we run a control tube containing exactly the same amount of the acid. Like the Kjeldahl titration, however, it is best to have such an acidity that each cubic centimeter of the control should not require more than 1 cc. of the standard alkali solution. That is, if one used the alkali solution to dilute the N/20 H_2SO_4 to 100 cc. with MR-MB, the original acidity should be slightly more than twice as strong as the standard alkali. If too strong acid is used, the indicator is so diluted during titration, that the end point may be a little obscure. If too weak acid is used, the acid may be already neutralized by the ammonia before titration. Thus according to the type of the tissue, length of the respiratory period and weight of the nerve, the acidity may have to be altered after a preliminary experiment. For ordinary 15 minutes' respiration with 2 sciatic nerves, a MB-MR-acid solution prepared in the following ratio is found to be satisfactory: 0.4 cc. N/20 H_2SO_4 + 1.2 cc. MB-MR are made up to 100 cc. with the standardized alkali.

D. Detailed method of estimation of the NH_3 given off by the nerve. A typical experiment to estimate simultaneously the amount of NH_3 given off by resting and stimulated nerve is as follows: Four clean test tubes are placed in the rack, each tube being provided with a paraffined cork, having 2 electrode attachments. Prepare the indicator-acid solution, by taking 0.4 cc. N/20 H_2SO_4 + 1.2 cc. MR-MB and making it up to 100 cc. with the standard alkali solution, using a flask and both pipettes made of Pyrex. By means of another Pyrex pipette, 1 cc. of each of the indicator-acid solution is placed in the above tubes, and tightly stoppered with the cork. Two pairs of the sciatic nerves are quickly isolated from two frogs, and immersed in each of two dishes containing Ringer's solution. One out of each pair is taken together, blotted and weighed. The remaining two are also likewise weighed. After the adhering liquid is carefully removed by means of filter paper, each set of nerves is placed in tubes *S* and *R*, respectively, the corks are replaced and stimulation by a weak induction shock is applied to the nerves in tube *S*, recording the time at which the respiration begins. The stimulus should be so weak as to be barely perceptible to the tongue. Tubes c_1 and c_2 are controls. All the tubes are shaken gently once or twice during experiment, care being taken not to let the liquid touch the nerves. The best way is to shake the rack gently without touching each tube with finger, in order to avoid temporary rise of temperature of the tube.

At the end of 15 minutes' respiration, each tube is shaken before the tissue is removed. After the stopper is removed, the upper third of the tube is wiped with filter paper so as to remove any liquid of the tissue which might have been left on the side of the tube.

The four tubes are placed in a basket which is hung in boiling H_2O, and which is placed at least one inch above the bottom of the beaker in which the water is boiling. At the end of 6 minutes, the tubes are removed and titrated while still hot with the standard alkali. The standard alkali can be introduced either from Pyrex buret, syphon or pipette, provided* we have standardized value of each drop of our alkali with the same apparatus.

The different amounts of drops of alkali required to neutralize control tubes and tubes containing the nerves when multiplied by number of grams of NH_3 equivalent to one drop of the alkali will indicate the amount of NH_3 given off by the nerves during that period of respiration.

The record given in table 3 will give an idea of the details of the method used in above experiments.[8]

TABLE 3

Exper. 8. 12/24/1920. Two frogs, Rana pipiens (♂, 22 cm.; ♂, 20 cm.) of lot VI (arrived at the laboratory on 11/12/1920) decerebrated simultaneously at 10:57 a.m. Respiration at 24°C.

TISSUE			NUMBER OF TUBES*	STIMULATION	RESPIRATION (15 MINUTES)		DROPS OF ALKALI NECESSARY TO NEUTRALIZE THE REMAINING ACID	AMOUNT OF ACID NEUTRALIZED BY NH_3	TOTAL NH_3 GIVEN OFF	NH_3 GIVEN OFF BY 10 MGM. OF THE TISSUE DURING 10 MINUTES
KIND	Isolated at	Weight of			From	To				
	a. m.	*mgm.*			*a. m.*	*a. m.*		*drops*†	*gram*	*gram*
2 sciatics	10:58	80	S	Yes	11:09	11:24	7	7	0.98×10^{-6}	0.81×10^{-7}
2 sciatics	10:59	80	R	No	11:09	11:24	11	3	0.42×10^{-6}	0.35×10^{-7}
		0	C_1		11:10	11:25	14			
		0	C_2		11:10	11:25	14			

* Each tube contains 1 cc. of MB-MR-acid solution.

† Each drop of the alkali is equivalent to 0.14×10^{-6} gram NH_3.

[8] Immediately after smoking, the breath contains appreciable amounts of base-forming substances. It is always safer, therefore, for a smoker to wash his mouth thoroughly before he uses pipettes.

RESULTS: *1. Resting nerve.* The results obtained by the last method show that the resting nerve gives off exceedingly small, but quite definite amounts of ammonia. The results given in table 4 were taken from experiments conducted with two bundles of sciatic nerves (70–100 mgm.) taken from frogs ranging in size of 19 to 24 cm. in length, under ordinary range of temperature variation (20–24°C.). The gas was collected exactly during 15 minutes of respiration starting it at about 10 minutes after the animals were decerebrated. The other nerves of the same frogs were stimulated and the NH_3 collected simultaneously with that of resting nerves.

In the following table, NH_3 production from stimulated and non-stimulated nerves are given to show the range of variation. If one compares two nerves of the same animal, we have no difficulty in showing increased NH_3 production during stimulation, but the actual amount of the gas given off by nerves of different animals varies considerably. Although these variations might be due to an error of the method which is necessarily great, yet we have evidence to show that there are other experimental and physiological conditions which have a great influence upon NH_3 production. An investigation is now under way in which these influences are more carefully studied. Until we shall know more about these factors, it is of prime importance to determine the amount of NH_3 given off by the unstimulated nerves of the same frog and compare it with the NH_3 produced by the other half under various conditions whose influence one wishes to investigate.

On the basis of 10 mgm. of the nerve for 10 minutes of respiration the unstimulated sciatic nerves of frogs (*Rana pipiens*) give 0.32×10^{-7} grams NH_3 on the average.

It is interesting to note that this amount of NH_3 corresponds to approximately $\frac{1}{17}$ of the weight of CO_2 given off under approximately the same conditions, and therefore for each mol. of CO_2, 1/6.5 of a mol. of NH_3 is given off, i.e., $\frac{1}{13}$ of equivalent weight of CO_2.

2. Stimulated nerve. In these experiments we used the ordinary method of electrical stimulation by weak induced current only, similar care being taken as described in our earlier work on CO_2 production. The remaining 2 of the nerves of the frogs, used in the experiment with the resting nerve were taken and stimulated side by side with unstimulated nerve, thus maintaining physiological and other experimental conditions as constant as possible.

The average amount of NH_3 by stimulated nerves is 0.68×10^{-7} grams as expressed on the basis of 10 mgm. tissue and 10 minutes'

respiration. Thus average amount of NH_3 given off during stimulation is approximately twice that of the resting nerve. This corresponds closely to the average increase of CO_2 in similar nerves during stimulation which was found to be 2.4 times that of the resting nerve.

TABLE 4

NUMBER OF EXPERIMENT	TEMPERATURE	WEIGHT OF NERVE	TIME ELAPSED FROM DECEREBRATION OF FROGS TO BEGINNING OF RESPIRATION	DURATION OF RESPIRATION	STIMULATION	TOTAL NH_3 GIVEN OFF	AMOUNT OF NH_3 GIVEN OFF, CALCULATED ON BASIS OF 10 MGM. OF NERVE AND 10 MINUTES OF RESPIRATION
	degrees C.	*mgm.*	*minutes*	*minutes*		*grams*	*grams*
2	23.0	105	9	15	−	4.0×10^{-7}	0.254×10^{-7}
		102	9	15	+	7.0×10^{-7}	0.457×10^{-7}
3	23.5	80	9	15	−	3.6×10^{-7}	0.30×10^{-7}
		80	8	15	+	10.8×10^{-7}	0.90×10^{-7}
4	20.0	90	11	15	−	4.8×10^{-7}	0.355×10^{-7}
		85	11	15	+	9.0×10^{-7}	0.705×10^{-7}
5	20.2	68	13	15	−	2.4×10^{-7}	0.235×10^{-7}
5a	20.0	95	13	19	−	5.6×10^{-7}	0.31×10^{-7}
		88	13	19	+	14.0×10^{-7}	0.83×10^{-7}
6	20.2	78	11	15	−	4.9×10^{-7}	0.419×10^{-7}
		72	11	15	+	7.0×10^{-7}	0.642×10^{-7}
8	24.0	80	11	15	−	4.2×10^{-7}	0.35×10^{-7}
		80	11	15	+	9.8×10^{-7}	0.81×10^{-7}
9b	23.0	80	13	15	+	4.8×10^{-7}	0.4×10^{-7}
Average for non-stimulated nerve							0.32×10^{-7}
Average for stimulated nerve							0.68×10^{-7}

3. NH_3 production following stimulation of the nerve. In these experiments, a series of test tubes containing exactly the same amount of MB-MR-acid solution was prepared in a group of 4. Two nerves were placed in the first tube and the other two in the next, the remaining two of the group acting as controls. The nerves in the first tube were stimulated for 15 minutes. At the end of 15 minutes, after usual procedure, both stimulated and unstimulated nerves were transferred into

corresponding tubes of the second group, no more stimulation being applied to the stimulated nerve during subsequent respiration.

The results of titration of each of these later tubes show that during the first 15 minutes following stimulation, the nerve gives off always more than the unstimulated nerve. Under ordinary condition, the amount of NH_3 produced by the unstimulated and the stimulated nerve during post-stimulation becomes equal at the end of 45 to 60 minutes. We have not yet succeeded in establishing the exact condition under which the two nerves give exactly the same amount of NH_3 after a definite time. One thing is certain, however, that an increase in NH_3 production due to stimulation keeps on for some time after the external stimulation is stopped.

Whether this increased NH_3 production during post-stimulation is due to an increase of NH_3 formation due to hyper-irritable condition of the nerve during post-stimulation, diffusion of preformed NH_3 produced during previous stimulation, or due to release of NH_3 by virtue of gradual oxidation of lactic acid, which if formed should tend to hold a part of NH_3 formed during stimulation, can not be decided without further experiments.

4. Effect of injury. We have shown before that the nerve, when mechanically injured, gives off more CO_2 than the uninjured, and attempted to explain the fact by considering the traumatic injury to be analogous to an extreme form of stimulation. Since a stimulated nerve gives off more NH_3 in a similar manner to CO_2, we expected to produce more NH_3 in the nerve. The results were, however, diametrically opposite. The crushed nerve not only does not give off more NH_3 than the resting, but gives far less, the amount varying from $\frac{1}{2}$ of that of the resting to none at all.

The mere fact that the nerve gives off less NH_3 under these conditions than uninjured and unstimulated nerve, does not necessarily, of course, rule out the possibility of an increase NH_3 production during trauma. On the contrary, in spite of these facts, there are reasons for believing that an actual formation of NH_3 is increased under all forms of stimulation.

Whether or not the nerves produce lactic acid under certain conditions has not been experimentally settled. If the nerve behaves similarly to muscle, then during trauma even if NH_3 might have been formed, we shall not be able to detect it by our present method unless such acid is removed by either being converted to a neutral substance or oxidized away. In case of muscle, however, Fletcher and Hopkins

(5) have shown definitely that the lactic acid formed during trauma can not be removed in the manner that happens to lactic acid produced during functional activity.

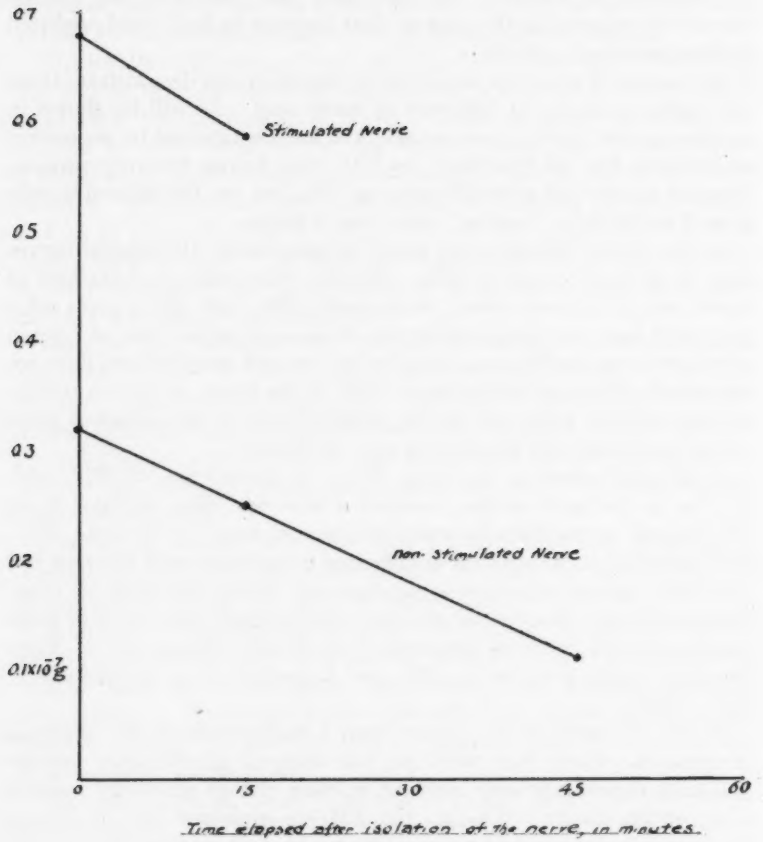
In the case of ammonia formation in muscle we can demonstrate these two different modes of behavior of lactic acid. As will be shown in another article, both injured muscle and muscle fatigued by successive stimulation give off practically no NH_3, but during recovery process, fatigued muscle will gradually give up NH_3, but not the injured muscle even if we let them "respire" more than 2 hours.

In the nerve, during a long period of respiration, the injured nerves seem to give up a part of NH_3. Whether this indicates formation of lactic acid in injured nerve, which holds NH_3, but which gives off a part of it later by partial oxidation of the acid unlike that of injured muscle or it means that traumatic injury was not complete and does not completely eliminate formation of NH_3 in the nerve, or that a certain amount of NH_3 keeps on coming independently of physiological process is a question to be determined more in detail.

5. Effect of standing on nerve NH_3. A knowledge of NH_3 production in the nerve during successive intervals after isolation from the animal is necessary in order to know the source of the gas. Gad-Anderson (6) has shown that muscle urea is spontaneously decomposed into NH_3 during post-mortem change, and certain bacterial or other decomposition is known to set in at a surprisingly early state of post-mortem period, such as histamine formation. Either one of these processes might give rise to NH_3 gas formation in a comparatively fresh tissue.

In general, isolation of a tissue from a body produces two opposing phenomena. From this point on, the survival physiological process gradually descends to zero, but post-mortem change gradually ascends to a certain climax. Whether the NH_3 we measured here is formed by survival physiological process or due to post-mortem phenomena unrelated to process in normal body, will be decided by the nature of the curve of NH_3 production during successive periods after the nerve is isolated from the body.

The gradual decrease of NH_3 production shown in the following chart, shows that NH_3 production in the nerve must be due to physiological process. We have not yet extended our observation on a large interval to see just when the physiological process reaches a minimum and to compare this point to the death point of the nerve determined by irritability and CO_2 method.



Curve 1

SUMMARY OF QUANTITATIVE RESULTS. The summary of quantitative estimation of NH_3 produced by the nerve under various conditions is given in table 5.

6. Quantitative results with nephelometer. In order to check the results obtained by the method based on base-forming property of gas, a nephelometric determination was made. N/800 H_2SO_4 is used to

TABLE 5

Summary of NH_3 production from the sciatic nerves of frog, Rana pipiens, under different conditions

CONDITIONS	RESPIRATION PERIOD	AVERAGE AMOUNT OF NH_3 GIVEN OFF, CALCULATED ON BASIS OF 10 MGM. OF THE NERVE AND 10 MINUTES RESPIRATION	TEMPERATURE
		grams	*degrees C*
Resting nerve.....	15 minutes immediately after*	0.32×10^{-7}	20–24
	Next 15 minutes	0.25×10^{-7}	20–24
Stimulated nerve ..	15 minutes immediately after	0.68×10^{-7}	20–24
	Next 15 minutes	0.59×10^{-7}	20–24
Crushed nerve.....	15 minutes immediately after	0 to 0.15×10^{-7}	20–24

* "immediately" is used for those cases when the respiration was started within 7 to 10 minutes after decerebration of the animal.

TABLE 6

NUMBER OF EXPERIMENT	TEMPERATURE	WEIGHT OF NERVE	TIME ELAPSED FROM DECERBATION OF FROGS TO BEGINNING OF RESPIRATION	DURATION OF RESPIRATION	STIMULATION	TOTAL NH_3 GIVEN OFF	AMOUNT OF NH_3 GIVEN OFF CALCULATED ON BASIS OF 10 MGM. OF NERVE AND 10 MINUTES OF RESPIRATION
	degrees C.	*mgm.*	*minutes*	*minutes*		*grams*	*grams*
AN 1	25.0	101	11	20	+	5.3×10^{-7}	0.26×10^{-7}
		103	11	20	–	3.8×10^{-7}	0.18×10^{-7}
AN 2	23.0	123	11	15	+	4.6×10^{-7}	0.25×10^{-7}
		141	11	15	–	3.8×10^{-7}	0.18×10^{-7}
AN 3	19.0	86	9	15	+	4.6×10^{-7}	0.35×10^{-7}
		87	9	15	–	2.8×10^{-7}	0.21×10^{-7}
AN 11	23.0	84	16	20	+	3.3×10^{-7}	0.20×10^{-7}
		87	16	20	–	2.6×10^{-7}	0.14×10^{-7}
AN 12	22.5	101	12	20	+	4.4×10^{-7}	0.22×10^{-7}
		90	12	20	–	2.6×10^{-7}	0.14×10^{-7}
AN 14*	24.0	103	12	20	+	6.4×10^{-7}	0.31×10^{-7}
		103	12	20	–	4.4×10^{-7}	0.21×10^{-7}
Average for non-stimulated nerve.....							0.17×10^{-7}
Average for stimulated nerve.....							0.27×10^{-7}

* Determined by Nessler.

absorb the gas, and treated with Graves' reagents as described on page 525. Both the Kober and our own modification of Dubosque colorimeter were used to determine resulting turbidity. On account of difficulty of the method, large numbers of readings were necessary for each experiment and our data are not extensive. The results obtained by this method are given in table 6.

In the sense of ordinary quantitative analysis, these two results shown in tables 4 and 6 cannot be said to be in a close agreement. Unfortunately like most so-called super-michrochemical methods, our method is subject to a much larger per cent of error than that which ordinary quantitative accuracy permits. It is highly probable that in our method there may be more than one error which is common to all our determinations. The absolute amount of NH_3 recorded here will no doubt be revised by some who will devise a more accurate method. In spite of this, however, we are quite certain that the relative amount of NH_3 gas produced by the nerve under various conditions will stand regardless of any method, within physiological variation.

Considering, therefore, the amount of the gas we are dealing with, these data obtained by methods based on entirely different chemical properties are close enough to show that the gas is ammonia.

CONCLUSION

However curious it may seem, the facts are that the nerve undergoes chemical reactions in which both acid-forming and base-forming substances are produced, and that the increase of CO_2 production during stimulation is accompanied with an increase of NH_3 production. Although the physiological and biochemical significance of these facts has not yet been investigated, they raise many interesting questions.

Where does NH_3 come from? We have endeavored to show that it is neither produced by bacterial decomposition nor from urea. Considering the minuteness of its amount, one might naturally suppose that inasmuch as the blood always contains a little NH_3, the nerve might receive it from the blood and retain it in an amount which will be in direct equilibrium with the blood, or lymph, and that this NH_3 will gradually diffuse out to a medium from which it is constantly removed. That this plausible explanation will not hold will be shown in later papers where we shall present evidence to show that NH_3 production from the different tissues is not the same, but varies within a large range, and that these variations are not proportional to anatomical variation, but due to some other physiological and biochemical factor.

Thus the process of elimination leads us to the speculation that this NH_3 must come from protein directly.

What becomes of it? According to the estimate made by Professor Donaldson,[9] an average adult human being weighing 150 pounds has 1,620 grams of total nervous tissues. If these tissues give off NH_3 in the same ratio as that of the sciatic nerve of the frog, we see that the daily output of NH_3 will amount to approximately 0.7 gram with corresponding variation during stimulation. Since the daily output of a normal man usually does not go beyond 1 gram of NH_3 and some other tissues also give off the gas in different degrees, and since by external factor alone one can almost completely abolish NH_3 output, even this entirely unqualified calculation tells us that the nerve NH_3 must be converted either entirely or partly into something else. It is not extraordinary speculation to consider that it is urea into which this ammonia is converted. Since no constituent of the urine is more variable than the urea, if NH_3 does go into urea, a variation of NH_3 production due to the changes in the nervous activity might easily be lost sight of.

How far this NH_3 influences acid-base balance in the body, and at what point it is converted to urea or something else can not even be speculated upon without further experimentation such as determination of the NH_3 production from the other tissues as well as an accurate determination of the NH_3 in the blood—a procedure which is one of the most difficult and unreliable of biochemical analyses.

What relationship has NH_3 with the irritability of the nerve? Does the fact that stimulation increases NH_3 production, suggest a decrease of NH_3 production during anesthesia? Muscle gives off far less am-

[9] Personal communication. For a man weighing 150 pounds, and who is 68 inches tall, his estimate of the total nervous system is as follows.

	grams
Brain	1400
Spinal cord	27
Sympathetic	30
Cranial nerves	12
Spinal nerves	151
Total weight	1620

Except weight of sympathetic which he considers more or less as a guess, these calculations are based on accurate anatomical data (8), (9), (10). For this information and many other suggestions, the author is greatly indebted to Prof. H. H. Donaldson.

monia than the nerve. Doctor Mathews suggested that this fact might be responsible for the extreme sensibility of muscle and relative immunity of the nerves to ammonia. Is there any relationship between NH_3 production and fatigue in view of the fact that the ratio between NH_3/CO_2 in activated nerve is far greater than that of the contracting muscle? What will be the NH_3 production from the nerve during prolonged refractory periods which are supposed to be more susceptible to a continued activity?

The accumulation of insoluble calcium salts at one point must be intimately concerned with a metabolism which forms bases. Drew (7) has shown that denitrifying bacteria can precipitate out $CaCO_3$ from sea water. In the physiological process of calcification and the pathological cases of softening of bone as well as pathological process of calcification, ammonia production in the tissues must be a dominant factor. This supports the idea that conditions like osteomalacia may be intimately related to disturbances in protein metabolism. Experiments to determine NH_3 production in the bone under different conditions will test these hypotheses.

In a series of papers on studies of alkaligenesis in the tissue, we hope to be able to answer some of these questions. Meanwhile we should emphasize the fact, as shown elsewhere (3), that any method which attempts to measure the small increase of CO_2 in the activated nerve should not ignore this exceedingly small but definite amount of NH_3 which is simultaneously formed in the nerve fiber.

SUMMARY

1. Evidence is given to show that resting nerves give off exceedingly minute quantities of a volatile base-forming substance which during stimulation is greatly increased, and that this substance is probably ammonia.

2. Methods are described by which we can measure NH_3 as small as 0.000,000,1 gram.

3. Quantitative data are given to show NH_3 production by the nerve under various conditions.

4. Various questions were raised concerning the rôle of NH_3 in the general physiological problem.

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STUDIES ON THE CONDITIONS OF ACTIVITY OF THE ENDOCRINE GLANDS

X. The Cardio-Accelerator Substance Produced by Hepatic Stimulation

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In a previous paper of this series (1) evidence was presented that stimulation of the hepatic nerves causes a substance to appear in the blood passing through the liver that accelerates the denervated heart and induces a rise of blood pressure. We wished to secure information regarding the nature of this pressor and cardio-accelerator substance.

In the experiments to be described, as in the earlier series, we used cats under light, but complete, ether anesthesia. The hepatic nerves were stimulated by means of shielded electrodes which prevented any spread of current. The strength of stimulus was approximately constant in all cases, and the stimulation lasted uniformly 30 seconds.

Further evidence that the cardio-accelerator agent is of hepatic origin. The reason for attributing the faster heart rate to a substance produced by the liver was that excitation of the hepatic nerves induced the change when all the extrinsic nerves of the heart had been severed and the only means of transmission between the liver and the heart was supposed to be the blood stream. The connection of the liver with the heart through the inferior vena cava was not considered. Might it not be possible that a disturbance induced in the hepatic veins could be carried along the cava to the heart and the rate thus accelerated? This possibility we tested by stimulating the cava low in the thorax. No effect on the heart was produced and therefore that possibility was excluded.

Again, if a substance is produced by the liver we should be able to collect blood containing it and, on injecting that blood, we should expect to produce the usual results of hepatic stimulation. To carry out this experiment we have introduced a small catheter, oiled inside and out,

into the right jugular vein low in the neck and down past the heart into the inferior cava nearly to the diaphragm. The cava below the liver was then closed (the adrenals had previously been removed), and after the hepatic nerves had been stimulated for 25 of the standard 30 seconds, 10 cc. of blood were drawn through the catheter into a syringe. The experiment is difficult to perform, and we have had a number of failures. In one instance, however, we succeeded in securing 10 cc. of blood (at 12:33). The heart rate, which had previously risen 22 beats per minute in consequence of hepatic stimulation, rose 24 beats (from 182 to 206 beats per minute) in consequence of stimulating the nerves at the time of withdrawing the blood from the liver viens. At 12:34 the rate had fallen to 196. The cava below the liver, which had been opened after removal of the blood, was now closed again and (at 12:34) the blood was slowly, during 15 seconds, reinjected into the thoracic cava. The heart rate rose from 196 to 208 beats per minute; a minute later the rate had fallen to 192, and shortly thereafter it returned to 180. In a control test 10 cc. of blood were withdrawn (at 12:51), without stimulation of the hepatic nerves; its reintroduction (at 12:53) was not attended by an increase in the heart rate. As will be mentioned later, there is evidence that an extract of blood which has been clotted, or blood which is approaching coagulation, will accelerate the heart. The brief period during which the blood was withheld in this positive experiment (1 minute) and the failure of an effect when no stimulation occurred, though the blood was withheld for 2 minutes, seem to us to rule out the possibility that the increased rate was due to blood changes.

In the earlier study of the effect of hepatic stimulation on the heart rate it was noted that a greater cardio-accelerator action was commonly associated with digestion (1). This might be due to the influence of some material which, taken from the intestines into the blood and normally removed as the blood passes through the liver, is not removed if the hepatic nerves are stimulated. Thus the faster heart rate would be due to a failure of the liver, when stimulated through its nerves, to protect the heart against a cardio-accelerator agent produced in the alimentary canal. Stimulation might act by disturbing the circulation or by affecting the liver cells. The hepatic nerves, according to Opitz, constrict and lessen the blood flow in the hepatic artery much more than in the portal vein (2). Might it not be true that reduction of the arterial blood supply interferes with hepatic function so that material absorbed from the intestines is not removed from the portal blood? This possibility was tested by closing the hepatic artery in a well-fed, digesting

cat; the heart rate was not increased. For example, on January 20, at 11:10, hepatic nerve stimulation for 10 seconds increased the heart rate 18 beats per minute; at 11:15, the hepatic artery was closed without changing the rate a beat; nevertheless, stimulation of the hepatic plexus during the exclusion of arterial blood accelerated the heart 25 beats per minute. Furthermore, we found (Feb. 28) that when the portal vein was closed, hepatic nerve stimulation, which previously (at 3:32) had hastened the heart rate 24 beats per minute, still (at 3:42) raised the rate by 15 beats. And on April 6, after excision of the small and large intestines, the spleen and the pancreas, and ligature of the cava below the liver and of the aorta above the renal branches, hepatic stimulation still had as great an effect as before, speeding up the heart by 16 beats per minute. The absence of any change on closing the hepatic artery, and the continuance of the accelerator effect though the portal vein was closed or the alimentary canal removed, we interpret as showing that the accelerating agent is elaborated in the liver itself and does not come directly from the intestines.

The inference just drawn received further support in three cases cited in a previous paper (1, p. 362). In two of them, to which we can now add four more, acceleration on stimulating the hepatic nerves occurred though the animals, which had been well fed, were not digesting, and in a third, an animal which had been much excited, it failed to occur though the alimentary canal contained much meat in various stages of digestion. Thus hepatic stimulation may be quite effective in the absence of material coming from the intestine and may be ineffective when intestinal contents are present. In other words, the liver, not the intestine, is the source of the accelerating factor.

FEEDING EXPERIMENTS. As was recalled above, the cardio-accelerator effect was observed in our previous study to be large in many of the animals which were actively digesting; but with a few exceptions (which we shall consider later) it was slight if the animals were fasting or in poor physical condition. This difference suggested testing the effects of different classes of food.

Carbohydrate feeding. As a rule, after potato has been fed shortly before the experiment or for several days previously, excitation of the hepatic nerves does not cause a faster heart rate than is observable when an animal has been without food for a day or two. The following results illustrate what we have noted:

February 28. Cat without food 2 days, fed potato 4 hours before operation. Stimulation of the hepatic nerves caused an increment of 8 beats per minute.

March 14. A repetition of the experiment of February 28; increment of only 4 beats.

March 17. Another similar experiment, with potato fed 3 hours previous to the operation. Increments of 6 and 8 beats.

March 28. Cat fed potato for 6 days preceding test. Hepatic stimulation caused increments of only 8 and 4 beats.

Stimulation of the hepatic nerves induces an increase of sugar in the blood (3). The foregoing experiments show that this effect does not influence the denervated heart. Other experiments, to be reported later, confirm this conclusion. The results of the foregoing tests do not indicate that carbohydrate feeding is favorable to a large cardiac acceleration of hepatic origin.

Fat feeding. Since the liver is supposed to desaturate the fatty acids and, by introducing double bonds, to prepare them for more ready metabolism (4), it might be assumed that the presence of plenty of fat in the blood would be favorable for the elaboration of material which would increase the heart rate. In our experience, however, feeding fat either continuously for several days or shortly before the experiment does not have an effect different from that observed in the fasting animal. For example, on March 21, an animal which had taken a meal of lard the night before and which was actively digesting it at the time of the experiment showed cardiac accelerations of only 8, 6 and 6 beats, respectively, on repeatedly stimulating the hepatic nerves. And on March 26, an animal which had been fed only lard for 5 days and was again fed on the morning of the experiment, so that it was actively digesting fat, showed no acceleration, or at most an increase of 1 beat per minute when the hepatic nerves were excited. Nothing in the evidence at hand points to fats being in any sense favorable to the development or to the efficacy of the hepatic agent that accelerates the heart.

Protein feeding. The figures recorded in the second column of table 1 in the previous communication on the effects of hepatic stimulation (1, p. 356) were derived from experiments in which the animals were digesting meat. The average acceleration of the heart in these instances was 15.5 beats per minute. The average acceleration in fasting animals, recorded in the first column of the table, was 5.2 beats per minute, an effect which, as just pointed out, corresponds to that seen after carbohydrate or fat had been fed. Our tests of protein feeding have yielded figures quite different from the negative results of feeding potato and lard. On April 5 a test was made on an animal which had

been fed for a week on all the lean meat it would eat and which had been given 100 grams of ground lean meat the previous afternoon. At the time of operation the intestines contained material undergoing digestion. Stimulation of the hepatic nerves caused increments of the heart rate as high as 12 and 14 beats per minute. On April 6 a similar experiment was performed with increments of 14 and 16 beats. After eating meat (3 hours before operation), on April 8, a cat showed accelerations of 26, 12 and 22 beats per minute. An animal given meat for 4 days before and also on the morning of the test (Nov. 22) had a heart rate faster by 13 and 14 beats after hepatic stimulation. The same stimulation (Dec. 22) in a cat digesting meat accelerated the rate in successive tests 32, 28, 20, 20 and 10 beats.

A modification of the experiments on protein feeding was tried in giving milk for some days and meat shortly before the operation. For example, on April 12, in an animal which had had milk for 3 days and which had eaten meat 4 hours previously, repeated stimulation of the hepatic nerves caused cardiac accelerations of 26, 12, 8 and 10 beats, respectively. And again, on April 14, in a similar experiment, the increases were 18, 16, 8, 10 and 12 beats. On the other hand, in two cases (April 13 and 16), milk-fed animals which were digesting meat, the maximal accelerations were 6 and 8 beats.

There have been five instances in which, though protein food was present in the alimentary canal and digestion had progressed, hepatic stimulation had very slight effects. In one of them, however, urethane was used as the anesthetic and perhaps determined the result—a possibility which we have not examined. In another, decerebration was tried, and when the hepatic nerves were stimulated, the blood pressure had fallen to 42 mm. Hg.—a level probably too low to permit the liver, which is sensitive to a deficient oxygen supply (1, p. 362), to respond. In the third the hepatic artery was found to have been for a long time closed. The fourth cat had been greatly excited before the experiment, especially while being anesthetized. In the fifth no possible explanation of the slight accelerations (4, 4 and 9 beats) can be given, unless it was that the animal had been fed liver instead of muscle—an improbable reason, we think.

From the foregoing evidence we draw the conclusion that as a rule the acceleration of the denervated heart on stimulating the nerves of the liver is more marked when the animal is digesting protein than when the animal has been fasting or subsisting on carbohydrate or fat.

THE EFFECT OF INJECTING AMINO ACIDS. The relation between protein feeding and the efficacy of hepatic stimulation, the fairly close chemical relation of adrenin to certain amino acids, and the demonstration by Barger and Dale that there are amines having sympathomimetic effects (5), suggested that the acceleration of the heart and the rise of blood pressure when the hepatic nerves are stimulated are due to a release of an agent, or agents, of this nature from the liver cells. It seemed possible that amino acids or amines, transported from the intestine to the liver, would there be removed from the portal stream by the cells and that when the cells are stimulated they would release these

TABLE 1

Effect of injecting mixed amino acids (except cystine) into small intestine

DATE	AMOUNT INJECTED	INCREASE OF HEART BEATS PER MINUTE FROM HEPATIC STIMULATION 30 SECONDS							REMARKS
		Before injection	Minutes after injection						
			5	10	15	20	25	30	40	
	cc.									
February 28	20	8			19	23		24		Fasting 48 hours
March 2	20	2	2	2	2	8		8	12	Fasting 36 hours
March 4	10	6, 4	10	10	6					Fasting 36 hours
March 7	10	6, 6	7	10	9	12	8	8		Fasting 24 hours
March 17	10	6, 8	8		14	10		8	2	Digesting potato
March 21	10	8, 6			2	10		2	6	Digesting fat
March 26	10	0	2.5			2		2		Digesting fat
March 8	10	8		10	6	6			8	Digesting potato
April 5	10	12, 14	10			10				Digesting meat

In the last four experiments the amino acid mixture was neutralized with sodium bicarbonate.

substances before having time to alter them or incorporate them. It was conceivable also that the faster heart rate was an expression of the specific dynamic action of certain amino acids in accelerating metabolism

The results of injecting into the small intestine a solution of mixed amino acids (except cystine) are given in table 1. The first experiment (Feb. 28) yielded such striking results that we thought we were about to make a big step forward. The second experiment was not wholly disappointing, but thereafter the results were so uncertain as to leave us no sound basis for a conclusion. As the table shows, the effects

(except in the first two experiments) were not notably different whether the introduction of the amino-acid solution was preceded by fasting or was accompanied by feeding carbohydrate, fat or meat. One might think that the amount injected was too small, especially in the later experiments; on one occasion, however, the night before the operation we gave by stomach tube 40 cc. of the solution to a cat which had been without food 48 hours, and introduced 25 cc. more in the morning shortly before anesthetizing, but we were unable by hepatic stimulation to accelerate the heart more than 6 beats per minute.

A variation on the foregoing experiments was made by use of the filtrate from a pancreatic digest of casein, containing presumably all the amino acids and some polypeptides. An injection of 0.1 cc. of this filtrate into the femoral vein increased the heart rate 10 beats per minute; a second injection, 1.0 cc., raised the rate from 178 to 208, i.e., 30 beats per minute. After 7 cc. of the digest had been injected into the small intestine, however, the heart rate was not altered to any noteworthy degree, nor was stimulation of the hepatic nerves made more effective. In another experiment the stimulating effect of the filtrate itself, when introduced intravenously was confirmed—1.0 cc. increasing the rate 20 beats per minute—but this effect, we must emphasize, should be distinguished sharply from an effect on the liver, such as appeared to be present in the experiment of February 28, table 1, and was revealed by a greater efficacy of hepatic stimulation.

On the supposition that the faster heart rate might in some manner be related to the specific dynamic action of protein, we have tested the effect of glycocoll. Lusk has reported that when it is given by mouth it increases the metabolic rate (6). We found, however, that an injection (during 10 minutes) of 10 cc. of 5 per cent glycocoll in mammalian Ringer into the femoral vein not only failed to increase the heart rate, but actually reduced it, and also reduced the influence of hepatic stimulation. A stimulation which, previous to the injection, had increased the rate 22 beats per minute had no effect immediately after the injection was completed and 6 minutes later caused an increment of only 6 beats. A second injection of the same amount during 16 minutes into a branch of the portal vein did not depress the rate to so great an extent, but, as before, it did not favor the action of the hepatic factor on the heart.

Alanine (1 and 2 cc., 5 per cent), cystine (1 cc., saturated solution), tryptophane (5 mgm. in 2.5 cc.), cystine (5 cc., 1 per cent), leucine (the same), asparagine (the same), glutamic acid (the same), aspartic acid

(5 cc., saturated solution), and phenyl-alanine (5 cc., 1 per cent), had either no effect or a negligible effect (a change of 2 beats) on the heart rate. Ammonia (1 cc., 1 per cent), introduced into the colon, if it acted at all, reduced the heart rate.

There is some evidence that tyrosine, closely related in chemical structure to adrenin, is capable of accelerating the heart. On April 14, injection of 2 cc. of a saturated solution in mammalian Ringer into the femoral vein caused an immediate rise of 5 beats (from 182 to 187) and within 3 minutes 5 beats more, to 192 per minute, where the rate remained for more than 10 minutes. Again on May 12, injection of 1 cc. of a saturated solution of tyrosine in mammalian Ringer increased the heart rate within 3 minutes by 15 beats per minute. Since tyrosine is more soluble in acid and alkaline solutions we tried such preparations, and with 2 mgm. were able to induce increases of 10 and 11 beats. But in no instance was there produced any greater effect of stimulating the hepatic nerves. Introduction in one case of 4 cc. of a 1 per cent tyrosine suspension into the colon, and in another case 2 cc. of the same suspension into the small intestine led to no change in the heart rate and to no increased action of the hepatic factor.

As is well known, tyramine in small amounts will raise blood pressure and in other ways act like adrenin. We found that when injected into a vein or into the intestine in small amounts it increased the heart rate remarkably but did not cause any considerable change in the efficacy of hepatic stimulation. Thus on November 8, after the introduction of 2 cc. of a 1 per cent solution of tyramine into the small intestine had raised the heart rate from 228 to 246 and later to 276 beats per minute, hepatic stimulation, which previously had increased the rate 8 beats, still increased it 8 and 5 beats. In order to induce a slighter acceleration of the heart rate from tyramine passing through the liver, and thus to permit, perhaps, a greater opportunity for action from any tyramine that might have been taken up by the liver cells and that might be released when they were stimulated, we tried introducing a smaller amount. On November 15, 10 cc. of a 0.002 per cent solution injected into the intestine raised the rate from 210 to 232 beats; the increment following excitation of the hepatic nerves, however, which previously had been 12 and 9 beats, continued thereafter 8 and 12 beats.

As stated in the first paragraph of this section, the concept which led us to undertake the observations on amino acids and amines was that these agents might be taken out of the portal blood by the liver cells during protein digestion and might be released when the nerves were

stimulated and thus influence the heart. In that case these substances should themselves act on the heart when introduced into the general circulation. This effect has been noted only in case of tryosine and tyramine. Furthermore, if our concept were correct, when these substances are introduced into the intestine there should be an increased effect on the heart from exciting the hepatic nerves. With the exception of the first two instances given in table 1, however, this effect has not been seen. It seems to us probable, therefore, that the hepatic agent is not an immediate product of digestion, but some material elaborated by the liver in the course of time.

EFFECT OF KNOWN OR SUPPOSED LIVER PRODUCTS ON THE DENERVATED HEART. Best known of the substances resulting from metabolic changes in the liver are glucose and urea. It has been claimed also that the liver produces and discharges catalase. Since our experiments led us to the conclusion that the accelerator agent is some substance elaborated by the liver cells, we decided to determine whether any of the known or supposed liver products could account for the effect on the heart of stimulating the hepatic nerves.

Glucose. Locke and Rosenheim noted that when the rabbit heart was perfused with oxygenated Ringer solution, the rate was considerably augmented by the addition of glucose to the solution (7). Stimulation of the hepatic plexus will cause a prompt liberation of glucose from the liver (3). It seemed possible, therefore, that the faster cardiac rate might be accounted for by a greater concentration of glucose in the blood going to the heart. The cardiac rates per minute at the times indicated and the increments of rate (in parenthesis) due to uniform repetitions of hepatic stimulation, before and after intravenous injection of glucose, are shown in the following records:

January 8. Cat's weight, 3.1 k. At 12.10, 1 cc. 5 per cent glucose injected during 13 seconds into femoral vein. No change in heart rate.

February 18. 11:44, 192 (6); 11:50, 189 (4); 12:23, 188 (1). At 12:27, 10 cc. 5 per cent glucose injected into femoral vein during 25 seconds. 12:32, 186 (10); 12:37, 188 (8). At 12:39, 20 cc. 5 per cent glucose injected. 12:44, 186 (10).

February 19. 11:23, 115 (7); 11:27, 124 (10). At 11:29, 10 cc. 5 per cent glucose injected into femoral vein. 11:31, 124 (12); 11:37, 128 (12); 11:44, 127 (11).

February 21. 11:24, 193 (9); 11:51, 209 (3). At 11:58, 10 cc. 5 per cent glucose injected into femoral vein. 12:02, 202 (6); 12:08, 206 (6); 12:15, 200 (2); 12:20, 200 (4).

March 24. Cat's weight, 3.5 k. 5:33, 198 (10); 5:40, 200 (6); 5:50, 196 (12). At 6:00, 5 cc. 5 per cent glucose injected into femoral vein. 6:03, 192 (8).

As the figures show, injection of glucose in an amount which would increase the content in the blood to a degree that might result from hepatic stimulation (e.g., March 24) failed to have any effect on the heart rate. And the injection of much larger amounts (10 or 20 cc. of a 5 per cent solution) in animals weighing about 3 kilos, likewise caused no noteworthy change. Furthermore, though the experiments of February 18 and 19 seemed to indicate that the glucose injections rendered hepatic stimulation more effective, the later experiments did not. We conclude, therefore, that the increments of heart rate due to stimulating the hepatic nerves are not the consequence of liberated glucose.

Urea. The liver is supposed to be the principal place for the formation of urea after absorption of amino acids from the intestine. Backman noted that when he perfused a rabbit heart with 2 per cent urea in oxygenated Locke's solution, the heart beat was increased in both amplitude and rate (8). Though there is no evidence that an output of urea from the liver can be induced by nervous stimulation, it seemed at least possible that discharged urea might account for the faster heart rate of hepatic origin. To test this possibility we injected urea into the femoral vein. The cardiac rates per minute at the times indicated and the increments of rate (in parenthesis) due to repeated stimulations of the hepatic nerves, uniform in strength and duration, before and after the injection of urea, are shown in the following records:

January 22. Cat, 3.7 k. 11:49, 204 (3); 11:54, 198 (0). At 12:12 injected into femoral vein during 10 seconds 2.25 grams urea in 4 cc. distilled water. Heart rate fell, rose and fell again, 194, 186, 184, 188, 200, 184, and at 12:24, 182 (0). At 12:29 injected during 10 seconds 2 grams urea in 2.5 cc. distilled water. Heart rate fell and rose, 182, 174, 176, 180.

April 13. Cat, 3.5 k. 3:17, 186 (6). At 3:23 injected into femoral vein during 2 minutes 10 cc. 5 per cent urea (0.5 gm.) in mammalian Ringer. No immediate change in heart rate. 3:26, 184; 3:30, 188 (8). Injected again, at 3:34, 10 cc. 5 per cent urea, but during 20 seconds. Heart rate dropped 18 beats per minute and quickly recovered. 3:35, 192; 3:38, 184; 3:41, 188 (0).

April 14. Cat, 3.1 k. 2:15, 188 (4); 2:23, 194 (4). At 2:33 injected into femoral vein during 70 seconds 10 cc. 5 per cent urea in warm mammalian Ringer. No change in heart rate, which was 188 beats per minute. 2:35, 188; 2:38, 185.

As revealed in these observations the effects seen by us in the cat, unlike those noted by Backman, show that urea has either no action on the rate of the isolated heart or a depressant action. Moreover, injection of urea solutions did not render hepatic stimulation any more efficacious in accelerating the heart.

Catalase. Burge has stated that, in consequence of hepatic stimulation, catalase is given off by the liver into the blood stream (9). He regards this as an agent for increasing the rate of oxidative processes in the body. Recent investigations have not shown any close relationship between the metabolic activity of organs, including the heart, and their catalase content (10). Granted, however, that Burge's claims are correct that nervous influences cause the liver to discharge catalase and that this ferment accelerates oxidation, the faster heart rate after hepatic stimulation might be explained. It seemed to us, therefore, that this possibility should be tested. We have tried the intravenous injection of catalase made from the liver, after the manner described by Battelli and Stern (11). The results were as follows:

February 5. At 11:50, 1 cc. catalase solution (1 cc. added to 10 cc. H_2O_2 produced in 10 minutes 25 cc. O_2, at 20°C. and 757.5 mm. Hg. pressure) injected into femoral vein; heart rate changed from 206 to 205 beats per minute. At 11:55, 2 cc. of the solution, change of cardiac rate, 204 to 206. At 12:03, 4 cc., rate, 202 to 203. At 12:20 both adrenal glands removed. At 12:22, 2 cc. catalase solution, rate 203 to 200. At 12:30, 4 cc., rate 196 to 182. At 12:38, 2 cc., rate 190 to 184. (Cardiac rate dropping after adrenalectomy.)

April 12. Cat, 3.5 k. At 12:11, 212 (26); 12:17, 212 (12); 12:25, 200, (8); 12:37, 188 (10). At 12:41, 5 cc. liver catalase in warm mammalian Ringer (1 cc. liberated 6 cc. O_2 from 20 cc. of 50 per cent H_2O_2 in 10 minutes) injected into femoral vein; heart rate changed from 186 to 184. 12:44, 184. At 12:47, 10 cc. catalase solution injected; heart rate unchanged, 184. 12:48, 188; 12:52, 188; 12:55, 180 (10).

The foregoing results give no support to the idea that catalase, increased in the blood stream, can cause acceleration of the cardiac rate. Even though hepatic stimulation may evoke a discharge of catalase from the liver, therefore, that would not explain the faster heart beat that occurs.

Bile. The failure to find any influence on the denervated heart of substances known or supposed to be given off by the liver into the blood stream led us to note the effect of injecting bile.

There was no encouragement in this experiment to lead us to make other tests.

November 22. Cat, 4.8 k. 2:24, 195 (13); 2:33, 194 (14); 2:36, 192 (4); 2:48, 188 (4); 2:55, 187 (0); 3:10, 188 (4). At 3:56. 0.75 cc. bile (taken from animal's gall bladder) injected into femoral vein during 8 seconds. Heart rate changed from 182 to 180 beats per minute. 3:59, 179; 4:01, 180. At 4:07, 1 cc. of bile injected with no change of heart rate, 184 before and 184 after.

THE EFFECT OF LIVER EXTRACTS. Since extracts of the adrenal medulla are capable of causing the changes produced by adrenal secretion, it seemed possible that likewise a substance might be extracted from the liver that would have the effects seen after stimulating the hepatic nerves. Our first attempts were directed toward obtaining an extract in Ringer solution. The liver was ground in sand and allowed to stand in the cold over night. In no cases did these simple extracts of the liver, thus delayed in being tested, induce any acceleration of the heart that was worthy of notice. We next turned our attention to the possibility of extracting by boiling in weak hydrochloric acid (0.4 per cent), and nearly neutralizing with sodium hydrate. The filtrate was then injected, with the following results:

January 19. Cat, 3 k. At 11:52, 2 cc. injected during 10 seconds; heart rate increased from 230 to 244 (14 beats per minute).

January 20. Same extract used January 19. At 12:03, 2 cc. injected in 11 seconds raised the heart rate 6 beats, from 234. At 12:07, 2 cc. of dissolved aqueous solution of the alcoholic precipitate of the extract was injected; rate unchanged. Later the same injection lowered the rate 6 beats. At 12:12, injection of 2 cc. of muscle extract, made by the method used in extracting the liver, increased the heart rate 6 beats, from 238. At 12:23, 3 cc. liver extract injected; rate increased 6 beats, from 240. Adrenals removed, 12:43. At 12:50, 3 cc. liver extract injected in 16 seconds; heart rate increased 6 beats, from 204.

January 21. Cat, 3.1 k., in poor condition. At 11:00, 2 cc. of the same extract used January 19, injected during 13 seconds, increased the heart rate 4 beats, from 176.

January 22. Cat, 3.7 k. At 10:22, 1 cc. of the extract used January 19, injected during 6 seconds, increased the rate 13 beats, from 171. A solution of the alcoholic precipitate of the extract had no effect; but the filtrate, evaporated and diluted with water, raised the rate 8 beats, from 174 (at 10:35). At 10:41, 1 cc. muscle extract, made as the liver extract was made, had the following effects on the heart beats in three repeated injections, +4, +1, −2. Thereupon, 1 cc. liver extract, at 11:35, accelerated the heart 15 beats per minute, from 175; and 3 cc., at 12:01, increased the rate by 39 beats.

January 25. Cat, 4.7 k. At 10:32, 1 cc. of the extract made January 19, raised the heart rate 26 beats, from 180. At 10:38, a fresh liver extract, made in the same manner January 24, raised the rate 28 beats from 178.

These results seemed very promising, and we thought that we had a substance which was peculiar to the liver in accelerating the pulse. It was unlike hepatic stimulation, however, in that it caused a fall of blood pressure instead of a rise. That was suspicious. In order to make sure whether liver extracts were peculiar in accelerating the denervated heart we made, on January 25, fresh extracts of liver, pancreas, intestinal mucosa, gastric mucosa, salivary gland and skeletal muscle.

In each case the tissue was minced, ground in sand, boiled in 5 parts of 0.4 per cent hydrochloric acid, nearly neutralized (while boiling) with sodium hydrate, and then filtered. Thus all the tissues were treated alike. When a uniform amount (1 cc.) of each of these extracts was injected (Jan. 26), the cardio-acceleration caused by each in succession was remarkably uniform. E.g., liver, 4 beats per minute; salivary gland, 5; gastric mucosa, 5; pancreas, 5; intestinal mucosa, 8; muscle, 4; liver again, 4. From these results we concluded that liver, extracted in the manner described, yielded no peculiar substance affecting the denervated heart. We were thus blocked from progress in that direction.

After these observations had been made we found that simple extracts of the liver in Ringer solution, if *fresh*, would accelerate the pulse. To obtain material we tied off with a strip of gauze the tip of one of the liver lobes and cut it away without any bleeding. It was then promptly weighed, ground in sand, mixed with the salt solution in the ratio of one part liver substance to five parts Ringer, and filtered through cotton. We found in our first test that 5 cc. increased the heart rate 6 beats per minute and caused also a rise of blood pressure. Extract of spleen made in the same manner increased the rate only one beat. Hepatic stimulation caused increments of 4, 6 and 9 beats. Here were effects of injection that corresponded closely with the effects of stimulation. Again the results seemed favorable to some explanation. On extending our tests and comparisons, however, we soon found that although extracts of spleen, intestinal mucosa and lymph gland, all made in the standard way, usually had less effect than liver extracts, a preparation of salivary gland was commonly most effective of all in speeding up the heart. E.g., on December 15, 5 cc. each of extracts of the following tissues (1 part in 5 of Ringer solution) increased the pulse rate as indicated: liver, 12 beats; spleen, 6; intestinal mucosa, 6; lymph gland, 8. On the other hand, when (Dec. 17) liver quickened the pulse 26 beats, and spleen 8, salivary gland quickened it by 44. Though this is true, stimulation of the chorda tympani nerve is not accompanied by any faster heart rate.

It may be that all these tissue extracts influence the heart by changing the blood. We have found that extracts of clotted blood prepared as were the extracts of tissues mentioned above produce cardiac acceleration, and further, that blood approaching coagulation has that effect. But in almost every instance, when the preparation of tissues or blood is injected, whether rapidly or slowly, the blood pressure is lowered.

This is different from hepatic stimulation. In a former paper (1, p. 359) reasons were given for regarding the rise of arterial pressure and the faster heart rate which result from hepatic stimulation, as both being due to an agent carried from the liver to the heart in the blood stream. If that reasoning was correct the agent must be different from these extracts—a peculiar substance, not only quickening the pulse but also raising pressure.

General considerations. We have hesitated to infer that the hepatic agent is a new internal secretion. In an earlier communication, presented when we had evidence that in some instances injection of amino acids into the intestines would render hepatic stimulation more effective (see p. 549), and when also we had noted the accelerator influence of tyrosine, we drew the tentative conclusion that the effects observed are probably not due to a true internal secretion produced by the liver, but to a discharge from its cells of amino acids, or amines, which are sympatho-mimetic in action (12). Further experimentation, however, as detailed above, has led us to the inference that the cardio-accelerator and pressor factor is not some digestive product let pass through the liver when the nerves are stimulated, or quickly freed from the hepatic cells under nervous stimulation, but that it is a material elaborated in the liver. A study of the action of known liver products does not permit us to attribute to any one of them the observed effects. After a survey of all the facts we do not at present see any other assumption to make than that a substance of special and unknown nature is discharged by the liver cells into the blood stream when the hepatic nerves are excited.

Unfortunately, our experiments have told us what the hepatic agent is not, but have given us almost no indication of what it is. In 1914, Berg (13) reported that in the liver cells of well-nourished animals (salamanders, rabbits) there are numerous small masses or droplets which are lacking in fasting animals, and that they appear when protein, but not when carbohydrate or fat, is fed. A positive test with Millon's reagent proved them to be protein in nature—differing from the protein of the cell in morphological characters and constituting a protein storage. In 1920, Stübel (14) confirmed Berg's results and found that this stored protein could be greatly reduced in the liver cells by injecting adrenalin subcutaneously. Since adrenalin mimics the action of sympathetic impulses and since the hepatic agent is liberated when the splanchnic sympathetics are stimulated, it is possible that the minute protein masses observed by Berg represent the stored cardio-accelerator

and pressor factor. In this connection the diminishing effect of repeated stimulation of the hepatic nerves in our experiments is of interest. This was a common, though not an invariable, observation. Examples of it are as follows: April 6, increments of 26–12–22–7–8–6 beats per minute; April 12, 26–12–8–10–10–8–8–4–8; April 14, 18–16–8–10–12–4–4; April 16, 8–0–1–3; May 6, with secondary coil 6 cm. out, 20–15–6–4–2, then with coil 4 cm. out, 16–10–2–4; November 22, 13–14–4–4–4; December 20, 10–11–7–6; December 22, 32–28–20–20–10. These reductions in the efficacy of hepatic stimulation could be explained on the assumption that the liver cells become gradually discharged of their storage of cardio-accelerator material and that they require more time than we gave them to elaborate it again.

Cannon and Mendenhall have noted that adrenalin injected into the circulation shortens the clotting time, if the blood is circulating through the abdominal organs but not if the flow is confined to the anterior part of the animal (15). Foster and Whipple have lately brought forward evidence that the liver is the actively productive source of fibrinogen in the body (16), and also that diets rich in animal protein favor a high level of blood fibrin as contrasted with fasting, or with diets of carbohydrate or fat. These observations suggest that the liver contains material favorable to blood clotting that can be brought out by stimulating the nerve supply of the organ. And it may be that this material or the blood altered by its presence induces a more rapid heart rate and a slightly higher arterial pressure. These are only indefinite hints, however, and further elaboration of them would be futile in the absence of pertinent observations.

SUMMARY

Stimulation of the inferior vena cava does not accelerate the denervated heart. The accelerator agent, appearing when the hepatic nerves are stimulated must, therefore, be conveyed in the blood stream.

The accelerator effect can be produced by reinjecting into the inferior vena cava blood drawn from the hepatic veins during stimulation.

The occurrence of a faster beat, though the hepatic artery or the portal vein is closed, proves that the acceleration is not due to failure of the liver, during stimulation, to protect the heart from an accelerator substance absorbed from the intestines.

Feeding carbohydrate or fat is without influence on the effectiveness of hepatic stimulation in evoking a faster beat of the denervated heart. As a rule the stimulation is most effective when meat or milk has been fed and the animal is digesting meat.

Intra-intestinal injection of mixed amino acids occasionally renders hepatic stimulation more effective, but the results are not constant.

Injection of glycocoll, alanine, cystine, tryptophane, cysteine, leucine, asparagine, glutamic acid, aspartic acid and phenyl-alanine into the femoral vein is without noteworthy effect on the heart rate. Tyrosine (saturated solution in mammalian Ringer) can increase the rate. But neither this intravenous injection nor introduction of tyrosine into the intestine causes hepatic stimulation to be more effective. The same is true of tyramine. The hepatic agent, therefore, appears to be not an immediate product of digestion, but some material elaborated by the liver in the course of time.

Of the known or supposed liver products neither glucose, nor urea, nor catalase, nor bile causes acceleration of the denervated heart.

Fresh liver extracts in mammalian Ringer solution or acid extracts accelerate the pulse, but so do similar extracts of other organs, and especially the salivary glands.

We conclude that a substance of special and unknown nature, which increases the rate of the denervated heart and raises blood pressure, is discharged into the blood stream when the hepatic nerves are stimulated. The possibility that this substance is related to the protein masses stored in liver cells (Berg) and discharged by adrenalin (Stübel) is considered.

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VASOMOTOR RESPONSES OBTAINED BY SLOWLY INTERRUPTED FARADIC STIMULATION OF THE THORACIC SYMPATHETIC NERVE

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In a previous paper (1) it was pointed out that stimuli produced by a tetanizing current of sufficient intensity to elicit any vascular reflex from the central end of the cat's thoracic sympathetic nerve, divided just above the diaphragm and composed chiefly of fibers destined for the splanchnic nerve, resulted invariably in a rise of blood pressure. This is just the opposite of the effect obtained from central stimulation of the splanchnic nerve or thoracic sympathetic trunk in the dog. Auer and Meltzer (2) never obtained a rise in blood pressure from stimulation of the dog's splanchnic nerve, but rather drops amounting in some cases to as much as 60 mm. Hg. Burton-Opitz (3) also found that the characteristic reflex from stimulation of the central end of the dog's thoracic sympathetic nerve was a marked drop in blood pressure and he regards the splanchnic nerve as the depressor nerve for the abdominal viscera. It was also concluded that the visceral afferent pathway producing vasomotor reflexes probably consists of relays of short fibers with synapses in the gray matter of the spinal cord.

Obviously, the results obtained by stimulation of the thoracic sympathetic nerve with a tetanizing current immediately suggested the investigation of the use of a slowly interrupted faradic current upon this nerve. Hunt (4) first called attention to the fact that weak stimulation of an afferent somatic nerve elicited a fall in blood pressure. Gruber (5) and Gruber and Kretschmer (6), working upon the somatic nerves of cats anesthetized with urethane, showed that weak faradic stimuli with four interruptions per second, resulted in a drop in blood pressure in each instance, and that stronger stimuli with one interruption per second gave a depressor response in 93 per cent of the experiments. These results upon the somatic depressor reflex were verified by Vincent and Ogata (7).

Technique. Full-grown cats were used in these experiments to avoid variation in results due to age. The animals were anesthetized with urethane injected intraperitoneally. The dosage of the drug administered was 3 cc. per kilogram of body weight. Each 3 cc. of the solution contained 0.75 gram of the drug.

After isolating the brachial nerves in one extremity and preparing them for stimulation of their central ends, the carotid artery was prepared for blood pressure tracing and the vagi nerves divided. A tracheal cannula was then inserted and connected with an automatic compressed air machine which gave an interrupted supply of air at intervals corresponding to the normal respiratory rate.

To the cylinder which interrupted the air supply we attached a fiber wheel into which brass pegs had been driven at definite intervals upon its circumference. Each peg successively made contact with a spring to the end of which was attached a platinum needle. As each peg depressed the spring, the end of this platinum needle made contact with a cup of mercury. This entire apparatus was connected into the stimulating circuit. When the circuit key was closed a single stimulus would result each time contact was made into the cup of mercury. We so regulated the speed of the revolutions of the wheel that the stimuli occurred twice per second.

After mass ligation of the vessels in the chest wall, the thorax was opened upon one side parallel to the course of the seventh or eighth rib. The splanchnic nerve was then isolated just before it pierced the diaphragm, ligated and divided distally. The filament representing the thoracic sympathetic trunk below the point where the splanchnic is given off was cut, as were the rami communicantes of the tenth dorsal to the thirteenth dorsal segments inclusive. The splanchnic nerve and the lower portion of the thoracic sympathetic trunk, with which it is continuous, could then be raised by traction on the ligature at the time of stimulation. The usual precautions were taken to protect the nerve when we were not stimulating.

Results. The results obtained in the experiments upon ten cats were used as the basis for this article. Five other cats were used in this investigation but only upon these ten were depressor responses obtained from slowly interrupted stimulation of the brachial nerves and therefore these serve as a control of our results.

However, in all fifteen cats stimulation of the thoracic sympathetic nerve by a slowly interrupted current of sufficient strength to elicit any reaction resulted in a pressor response. As will be seen in figure

1, this response is similar to that obtained by the use of a tetanizing stimulus. This tracing also shows the typical depressor reaction resulting from stimulation of the brachial nerves with a slowly interrupted current of the same strength.

In none of the animals experimented upon was a drop in blood pressure obtained from slowly interrupted stimulation of the central end of the thoracic sympathetic nerve.

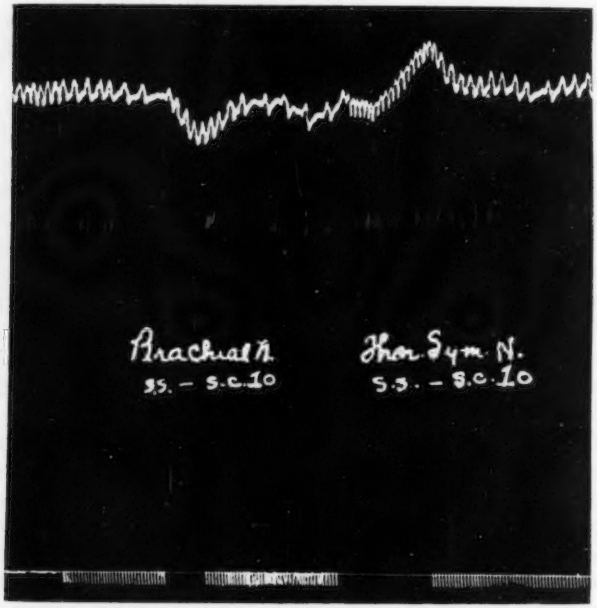

Fig. 1. Tracing showing the blood pressure response from stimulation of the central ends of the brachial and thoracic sympathetic nerves of the cat with a slowly interrupted faradic current.

Discussion. In the dog, stimulation of the central end of the vagus usually causes a pressor response, while in the cat the same stimulus causes a drop in blood pressure. On the other hand, the dog's splanchnic nerve gives a reflex resembling that obtained from the depressor nerve, while even with slowly interrupted stimuli the cat's splanchnic nerve is able to produce only a pressor reflex. This would seem to indicate that the connections which these nerves have with the vasomotor centers is radically different in these two mammals.

Whereas in the cat a somatic nerve may yield pressor or depressor reflexes under appropriate stimulation, the thoracic sympathetic nerve of this animal under these same conditions elicits only a pressor response. It will be recalled from the work of Ranson (8) that the somatic pressor path consists of short fibers in the tract of Lissauer and the apex of the posterior horn and that these chains of short neurons undoubtedly have many synapses. Very strong stimuli or rapidly repeated stimuli of moderate strength are necessary to break down the high synaptic resistance in the somatic pressor path. On the other hand we have shown that the visceral afferent pathway probably consists of relays of short fibers with many synapses in the gray matter of the spinal cord, yet those synapses are broken down by a slowly interrupted weak stimulus resulting in a pressor response.

CONCLUSIONS

1. Central stimulation of the lower end of the thoracic sympathetic nerve of the cat by a slowly interrupted faradic stimulus elicits a rise in blood pressure, although a similar stimulus of the brachial nerves causes a drop.

2. Stimulation of the central end of the dog's splanchnic nerve yields a depressor reaction while stimulation of the same nerve in the cat elicits a pressor response.

3. The synapses in the visceral afferent pathway of the spinal cord of the cat are easily broken down by slowly interrupted faradic stimuli of moderate or weak strength resulting in a pressor response.

4. Slowly interrupted faradic stimulation of the brachial nerves of the cat results in a depressor response which corroborates the work of Gruber.

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THE ISOLATION OF A SUBSTANCE FROM URINE HAVING PROPERTIES OF CITRIC ACID: DESCRIPTION OF AN APPARATUS FACILITATING THE WORKING WITH SMALL VOLUMES OF GAS

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The normal urine of human beings yields pentabromacetone when treated by the method used by Kunz (11) for the determination of citric acid in wines. Briefly the method is: Potassium bromid and sulfuric acid are added to urine and the mixture heated to just below 55°C. A solution of potassium permanganate is added slowly. When the reaction is completed the solution is cleared with ferrous sulfate. A crystalline precipitate of pentabromacetone is deposited. The method is fairly accurate for quantitative determinations of citric acid, if a correction is applied. Amberg and McClure (2) found that if a volume of 50 cc. is used it is necessary to add about 5.5 mgm. citric acid ($C_6H_8O_7.H_2O$) to the end result. Urine also gave a positive test when subjected to the treatment used by Salant and Wise (14) for the demonstration of citric acid in urine. This treatment makes use of the Denigès reaction (6), (7). A solution of citric acid is heated after the addition of a solution of mercuric sulfate in sulfuric acid. The addition of potassium permanganate drop by drop to the hot solution causes the formation of a white precipitate. Acetonedicarboxylic acid, formed by the action of the potassium permanganate on citric acid, in the presence of nascent bromine is transformed to pentabromacetone while in the presence of mercuric sulfate it forms an insoluble mercury compound. The results of Amberg and McClure indicated the presence of a substance in urine which readily yields acetonedicarboxylic acid. None of the substances hitherto known to occur in urine had been shown to possess this property. Because acetonedicarboxylic acid is a characteristic product of citric acid cleavage and because citric acid, a normal constituent of milk of the human being, is not foreign to

the organism, it was concluded that the acetonedicarboxylic acid was derived from citric acid. The proof was not absolutely unquestionable and the present study was undertaken to substantiate further the conclusion by the isolation of citric acid with the formation of characteristic salts and by elementary analyses. In this we were not successful, but the results of our experiments support the conclusion that citric acid is a constituent of the urine of normal human beings. After a number of preliminary experiments the following procedure for the attempted isolation of citric acid was adopted.

If the urine is strongly acid its acidity is reduced to weakly acid reaction (litmus) with sodium hydroxid. Lead acetate is added to slight excess and then ammonium hydroxid to strongly alkaline reaction. The mixture is repeatedly stirred for about 12 hours when the precipitate is filtered off and washed by being taken up in water alkaline with ammonium hydroxid. The filtrates are evaporated over a free flame to convenient volume; the reaction becomes acid during evaporation. The solution is freed from lead by treatment with hydrogen sulfid and the filtrate and washwater from the lead sulfid is evaporated to a syrup, with loss of the excess hydrogen sulfid. Some crystalline material separates which is filtered off and freed from adhering mother liquor by washing with ice-cold water. The precipitate is discarded. Barium acetate (30 per cent) is added to the thin syrupy solution until no more precipitate is formed. Sometimes barium hydroxid was added to weakly alkaline reaction, but this was not found to be of special advantage. After the addition of 95 per cent alcohol, two to three times the volume, the precipitate is allowed to settle. The supernatant fluid is syphoned off, the precipitate is repeatedly washed with 50 per cent alcohol, and is finally collected on a filter. The precipitate is extracted two or three times with hot water acidified with ortho phosphoric acid, and filtered off after each extraction. The filtrates are evaporated to a small volume and treated with moderate excess of sulfuric acid. Plaster of Paris is added, and the evaporation continued to dryness. The residue is divided as finely as possible with a spatula and extracted with ether in a Soxhlet apparatus, in which the ether is renewed several times. The ether extract is evaporated and the residue taken up in water and filtered from some sticky material which is discarded. The filtrate contains phosphoric and sulfuric acids. Therefore, lead carbonate is added in as slight excess as possible, the liberated carbon dioxid is boiled out and ammonia is added to make the solution about 2 per cent. The mixture is left

standing a day or more, repeated shaking being necessary, or it is heated with ammonia, filtered, and the precipitate taken up several times in ammoniacal water and filtered. On evaporation the solution becomes acid, and it is free from phosphoric and sulfuric acids. The lead is removed with hydrogen sulfid and the filtrate from the lead sulfid is evaporated to dryness. The residue is redissolved in water and filtered from a small amount of undissolved material. The color of this solution is usually light yellow; sometimes it is rather highly colored. If this is the case, it is heated with a small amount of animal charcoal. This is to be avoided if possible, since animal charcoal absorbs citric acid. The strongly acid solution is neutralized approximately with sodium hydroxid and treated with barium acetate solution until no more precipitate forms. The precipitate is collected and washed with graded dilutions of alcohol. This precipitate is the crude product.

From an accumulation of urine 6.8 grams of this crude product were obtained. Calculated from the amount of pentabromacetone obtained from part of it, it contained at least the equivalent of 1.6 grams of citric acid; that is, about one-half of it may have been barium citrate.

For further purification the crude product is again extracted with water acidified with phosphoric acid. The filtrates are evaporated to a small volume and 5 per cent solution of mercuric sulfate in sulfuric acid is added until no more precipitate is formed. The precipitate is discarded. Mercury is removed from the filtrate by treatment with hydrogen sulfid, excess of which is removed by boiling, the mercuric sulfid is filtered off and the filtrate is treated with lead carbonate as before. On evaporation, the ammoniacal filtrate becomes acid and a small amount of white precipitate separates. This is collected separately (A), and separated from the filtrate (B). (A) is washed well with water, taken up in water, and treated with hydrogen sulfid. The filtrate is freed from hydrogen sulfid by evaporation and the residue is taken up in water. On the addition of barium chlorid to the acid solution, some precipitate forms. Barium chlorid is added very carefully until the solution does not give any further precipitate, nor react with sulfuric acid. The precipitate is filtered off, the filtrate evaporated to dryness and the residue extracted with alcohol. On evaporation the alcoholic solution becomes a syrup which leaves very little residue when taken up in water; the residue is removed by filtration. To the strongly acid solution barium acetate is added. A snow-white precipitate forms, dissolves, and reforms on addition of more barium acetate, as happens on addition of barium acetate to citric acid.

Dried in a desiccator over sulfuric acid this white precipitate obtained from the crude product weighed 101.8 mgm. On drying at 163°C. it lost 7 mgm. of water. Ninety-four and eight-tenths milligrams of the dry substance yielded on analysis 79.5 mgm. barium sulfate which corresponds to 89.7 mgm. of dry barium citrate and it gave an equivalent amount of pentabromacetone.

The bulk of the filtrate (B) from the lead carbonate is evaporated further and more precipitate forms. The lead is removed from the precipitate suspended in the solution and the filtrate is evaporated to a small volume. A solution of barium chlorid carefully is added until no more precipitate forms. The filtrate from this precipitate is evaporated to dryness, taken up in water, filtered from a small residue and precipitated with barium acetate. After the precipitate was washed with water, dilute alcohol and strong alcohol, it weighed approximately 0.6 gram.

There is scarcely a step in the procedure which does not entail some loss, a conclusion drawn from the positive Denigès' reactions of the filtrates and washings. The substance used for the analyses was not quite pure, but we decided to use it rather than to lose it all by further efforts at purification.

It is known that citric acid heated with strong sulfuric acid gives off carbon monoxid, molecule for molecule. If the heating is done in a current of carbon dioxid, the carbon monoxid can be collected over potassium hydroxid permitting a quantitative determination of citric acid (16). If a substance gives pentabromacetone and carbon monoxid in equivalent amounts, it may be assumed that this substance is citric acid. The carbon monoxid determination was carried out as follows:

The dry substance is placed in a small Erlenmeyer flask and is moistened with water. The flask is closed with a three-hole rubber stopper, connected by one glass tube to the carbon dioxid generator, and by another to a gasometer charged with 50 per cent potassium hydroxid. From a small separatory funnel, inserted through the stopper, concentrated sulfuric acid is dropped into the flask which is then heated on a water bath over an asbestos plate; the liberated gases pass into the gasometer with the current of carbon dioxid. One to two hours after the collection of the gas, the volume is read; the gas is then transferred to an absorber filled with ammoniacal cuprous chlorid solution, and the carbon monoxid absorbed. The gas remaining is transferred to a measuring apparatus filled with water, in order to determine the necessary correction.

For our purposes an apparatus was devised to facilitate the handling of small volumes of gas without loss (fig. 1). The gas liberated in the reaction is collected in a nitrometer over 50 per cent potassium hydroxid. When the gas is to be transferred to the absorber, the capillary glass tube adjoining the stopcock of the nitrometer is filled with water. A short gasometer is fitted to this glass tube with a rubber stopper. Leading off from a small trough at the bottom of this gasometer is a side tube to which the rubber tube of a levelling bulb is attached. This gasometer is filled with ammoniacal cuprous chlorid solution from the levelling bulb, which is then lowered. The gas is transferred to the absorbing gasometer with the levelling bulb of the potassium hydroxid gasometer. The apparatus is sufficiently elastic to permit considerable shaking. When the absorption is complete a second short gasometer filled with water can be attached, and any remaining gas can be transferred and the volume read. The apparatus could easily be modified to meet special demands.

Fig. 1. Apparatus devised for the determinations of small amounts of carbon monoxid.

For the determination of carbon monoxid 0.1736 gram of the air dry barium precipitate was used, giving 0.0107 gram of carbon monoxid, an amount equivalent to 80.3 mgm. of citric acid (calculated $C_6H_8O_7.H_2O$). In determinations of pentabromacetone the Gooch crucibles containing the pentabromacetone precipitates were weighed after drying 24 hours in a desiccator over concentrated sulfuric acid. The pentabromacetone was washed from the crucibles with acetone, the crucible was dried and weighed again and thus possible errors from acetone insoluble impurities were avoided. For this determination 0.143 gram of the purified substance was taken; the yield of pentabromacetone was equivalent to 65.3 mgm. of citric acid.

It has been stated that the barium precipitate does not represent pure barium citrate. Before preparing the pentabromacetone the barium was precipitated and 111.6 mgm. barium sulfate were obtained. This amount is equivalent to 136 mgm. barium citrate, with $3\frac{1}{2}$ molecules water of crystallization. The pentabromacetone obtained corresponds to 132.6 mgm. of barium citrate, while 143 mgm. of the barium precipitate was used. The carbon monoxid is equivalent to 163.1 mgm. barium citrate, while 173.6 mgm. of the barium precipitate was used. The amount of citric acid which should be present in 173.6 mgm. of the barium precipitate calculated from the amount of pentabromacetone found in 143 mgm. of the substance is 79.3 mgm., while with the carbon monoxid method 80.3 mgm. were found. The remaining part of the barium precipitate was dried at 164°C.; 119 mgm. yielded 8.16 mgm. carbon monoxid, an amount equivalent to 61.2 mgm. of citric acid; 127 mgm. yielded an amount of pentabromacetone equivalent to 63.2 mgm. citric acid. The amount of citric acid to be expected in 119 mgm. of material is 59.3 mgm. calculated from the pentabromacetone obtained from 127 mgm., while with the carbon monoxid method 61.2 mgm. were found.

Some test experiments with barium citrate dried at 164°C. are tabulated:

	BARIUM CITRATE	CITRIC ACID	
		Calculated	Determined
	mgm.	*mgm.*	*mgm.*
Pentabromacetone method	177.6	94.4	93.9
	191.5	101.8	101.5
	170.7	90.8	89.7
Carbon monoxid method	131.3	69.8	70.4
	109.8	58.4	58.2

The results obtained with the material isolated from urine show a rather good agreement between the amounts calculated and those found, taking into consideration that the pentabromacetone method may give values a little low and the carbon monoxid method values a little high. It is questionable whether the result of the second determination, not so good as that of the first, is owing to errors of method entirely. Our substance was not quite pure, and while pure barium citrate does not change when water of crystallization is driven off, an

impurity in our material may have undergone some change; which might account for the discrepancy in the results.

Our material was sufficiently free from organic impurities that on heating with concentrated sulfuric acid the color remained pale yellow, as in control experiments with citric acid or citrates. With material less pure the fluid becomes dark or even black.[1]

The results were obtained with material from one accumulation of urine; not enough material was obtained from other accumulations for similar analyses, because efforts at purification resulted in loss.

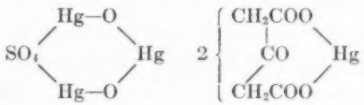
Only a few efforts were made to isolate our substance in the form of a calcium salt. It was very difficult to obtain any crystalline precipitates and if such occurred they were insignificant in amount. The difficulties here are as great as those encountered in efforts to isolate lactic acid from urine as a zinc salt. When calcium carbonate was added in place of barium acetate and the carbon dioxid was removed by heat, the neutral filtrate became acid and a small amount of a heavy crystalline precipitate separated rather early in the evaporation. This precipitate gave pentabromacetone and a positive Denigès' reaction. In one instance about 90 mgm. of this precipitate was obtained.[2]

With a small amount of good material the reaction of Sabanin-Laskowsky was carried out. The barium was carefully removed with sulfuric acid; a slight excess of ammonia was added to the filtrate which was evaporated. The dry residue was taken up in strong ammonia. The solution was placed in a sealed tube and heated to 134°C. for six hours as recommended by Scheibe (15). After cooling the solution was taken from the tube and left standing in the air, whereupon a blue color developed. The reaction was positive. Henkel says that this reaction is given by citric and aconitic acids but the latter does not yield pentabromacetone under the conditions of our experiments.

[1] The impurities included in such specimens may also give rise to carbon monoxid, and account for the discrepancy between results of the two methods. For instance, a specimen dried at 164°C. weighing 141.5 mgm. gave pentabromacetone corresponding to 54 mgm. citric acid or 101.7 mgm. of dry barium citrate. The carbon monoxid method indicated 87 mgm. citric acid in 159.2 mgm. of this specimen, while the calculation, from the pentabromacetone determination gave 60.8 mgm. of citric acid. The mixture became black when some of the substance was heated with sulfuric acid.

[2] The fact that this precipitate formed at acid reaction made us think of dicalcium citrate as obtained by Henkel (8) from milk, but the specimen dried at 100°C. gave 27.2 per cent calcium oxid on burning; dicalcium citrate yields 24.4 per cent, and tricalcium citrate 32.55 per cent.

Several substances such as aconitic, itaconic and citraconic acids yield a positive Denigès' reaction, although they do not behave exactly as citric acid does in the course of the reaction. It is not certain that precipitates from these acids contain acetonedicarboxylic acid, particularly, since they do not yield pentabromacetone. Nevertheless, precipitates obtained from our substance by the method of Denigès, treated with hydrogen sulfid, and extracted with ether, on evaporation of the ether left a dry residue which in water solution gave a purple color with ferric chlorid and a positive Legal reaction for acetone. The ferric chlorid reaction could not be obtained if the watery solution had been boiled first; by boiling, acetonedicarboxylic acid is decomposed to carbon dioxid and acetone. Some efforts were made to isolate pure acetonedicarboxylic acid from such precipitates. Denigès reported that it is possible to obtain pure acetonedicarboxylic acid from citric acid by way of the mercury compound. The proof of the purity of this acetonedicarboxylic acid rested on its mercury content.[3] A mercury compound made with acetonedicarboxylic acid prepared by the method of Pechmann contained the same amount of mercury. The formula as signed by Denigès to the mercury compound of acetonedicarboxylic acid is:



It is very doubtful whether the mercury compound of acetonedicarboxylic acid is of uniform composition. Salant and Wise (14) found it necessary to construct a table based on analytical results which gives the weight of the mercury compound obtained from different amounts of sodium citrate under definite conditions. With the aid of this table and within its limits, quantitative determinations of citric acid in aqueous solutions could be made. On repeating the experiment of Denigès we were able to obtain crystalline residues from the ether extract which gave the reactions described by Denigès, but the melting point of these residues varied from about 117 to 125°C. and were associated with decomposition. Denigès did not give the melting

[3] These precipitates when dissolved in dilute hydrochloric acid, and shaken with ether give up to the ether a mercury compound. This property may perhaps be used for purification.

point of acetonedicarboxylic acid prepared by him, and Pechmann (13) gives for the pure acid a melting point of 135°C. with decomposition.[4]

The mercury compound obtained from our substance gave precipitates the consistency and color of which varied from yellow and waxy to white and crystalline. Some of the crystalline precipitates contained white needles, and the melting point varied from 117 to 120°C. with decomposition. There was a great similarity between the properties of the ether residues obtained from our substance and those obtained from citric acid.

The work of Amberg and McClure (2) was undertaken in order to study the fate of citric acid introduced into the organism under various conditions. It was expected that some information would be gained concerning oxidative processes and their disturbances in the organism, but it was not expected that information concerning the oxidative power of organisms in general would be obtained. Dakin (4) very justly says, "The specific character of animal oxidations is most remarkable especially when phenomena such as those presented by diabetes and alcaptonuria are concerned. In these conditions oxidation of a readily oxidizable product of metabolism (glucose, homogentisic acid) may be completely restrained without impairing in the least the capacity of the body for effecting the oxidation of other substances." Batelli and Stern (3) working with animal tissues have shown that the oxidation of various organic acids proceeds under various conditions. Dakin's very interesting review of physiologic oxidations (5) makes the conclusion nearly unavoidable that the organism can bring about oxidations by various mechanisms. The excretion of readily oxidizable substances is of great significance in the light of such considerations, whether these are introduced from without or formed in the organism as an intermediary product of metabolism. The total amount excreted is of importance but of no less importance are the variations in the excretion of the individual acids. It may be suggested that a search for alcohols and aldehydes should be productive of results in certain conditions.

When salts of the lower aliphatic acids are present in sufficient concentrations they may exercise an influence on numerous biologic reactions. Among these are chemotaxis (Wolf, 17), phagocytosis (McJunkin, 12), and enzyme action (Amberg and Loevenhart, 1). Jobling, Eggstein and Petersen (19) showed that citrates particularly have an accelerating influence on tissue and serum esterase.

[4] Crude acetonedicarboxylic acid is readily prepared by the method of Pechmann, by the modification by Koessler and Hankel (10), or by the method of Denigès, but the purification is rather difficult.

In such reactions the individual acid exercises a more or less specific function:

In summarizing it may be said that the substance isolated from urine in the form of an impure barium compound had the following properties in common with citric acid.

1. It gave the pentabromacetone and the typical Denigès reactions.
2. It yielded pentabromacetone and carbon monoxid in amounts closely equivalent.
3. It did not char on heating with concentrated sulfuric acid.
4. It gave the Sabanin-Laskowsky reaction.
5. Precipitates obtained according to Denigès' method behaved very similarly to precipitates from citric acid. From these precipitates a dry ether residue could be obtained, the aqueous solution of which gave the characteristic color reaction for acetonedicarboxylic acid with ferric chlorid.

The results support our contention that citric acid occurs in the urine of normal human beings, but still, they do not convey absolute proof, which rests solely on the isolation of the acid or its salts in a form pure enough for identification.

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THE INFLUENCE OF MEAT UPON PHYSICAL EFFICIENCY

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The mystery regarding the significance of meat in the dietary is yet to be clarified. In the following experiments there were four different periods of one week in length. During the first week the usual normal diet was taken by each individual. During the second week a luncheon containing 300 grams of beef was served, with bread, butter and boiled potatoes, in the laboratory. This quantity of meat is the amount which was contained in the daily war ration of the French and Italian soldier. During the third week little or no meat was taken, and during the fourth week the same procedure was followed as in the second week. The four individuals engaged in this work analyzed their urines daily for nitrogen; all wore pedometers for measuring the number of their daily movements. They also were instructed to walk a given distance both morning and late afternoon on their way to and from the college, striving to accomplish the walk in the shortest time possible. On completing the walk the pulse was counted and then the time noted until the pulse rate became normal once more. This has been suggested by the work of Lewis, Cotton and Rapport (1) as a test of physical fitness.

The subjects were an instructor in the laboratory (R.), a Philippino (S.) working for a higher degree, a man (B.) and a woman (H.) student. The first named was in perfect physical condition after a summer in the Maine woods; the fourth was fond of long walks and tennis in which she indulged during the experimental periods. The other two subjects were in good general health. The basal metabolism of S., who weighed 40 kgm., was 41 calories per square meter of surface, the normal value.

The observations may be thus summarized:

TABLE 1

Subject B., weight 64 kgm.

	FIRST WEEK		SECOND WEEK		THIRD WEEK		FOURTH WEEK	
	Ordinary diet		High protein		Low protein		High protein	
Average number of steps per day	14,253		12,636		14,840		13,616	
Urine N (average) grams	13.14		17.44		8.68		18.34	
Maximum-minimum	14.08–11.31		18.57–17.12		11.37–7.53		21.67–15.48	
	a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk	11.43	12.4	12.7	14.5	14.0	17.0	16.5	15.0
Time in minutes to make 1000 steps	8.51	8.50	8.80	9.70	9.33	9.50	9.45	9.38
Pulse:								
Before exercise	71.0	71.0	73.0	75.0	73.0	74.0	77.0	79.0
End of exercise	86.0	85.0	102.0	101.0	100.0	93.0	107.0	102.0
After 3 minutes					87.0	80.0	91.0	88.0
After 5 minutes	72.0	75.0	86.0	79.0	84.0	77.0	88.0	84.0

TABLE 2

Subject H., weight 58 kgm.

	FIRST WEEK		SECOND WEEK		THIRD WEEK		FOURTH WEEK	
	Ordinary diet		High protein		Low protein		High protein	
Average number of steps per day	21,520		21,503		19,691		20,880	
Urine N (average) grams	9.64		14.72		8.02		16.91	
Maximum-minimum	10.43–8.80		17.06–13.34		8.78–6.86		19.38–15.83	
	a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk	26.0	27.0	26.0	27.0	26.0	27.5	26.0	27.5
Time in minutes to make 1000 steps	7.0	7.3	7.0	7.3	7.0	7.4	7.0	7.4
Pulse:								
End of exercise	112.0	107.0	120.0	105.0	121.0	117.0	113.0	114.0
After 4 minutes	78.0	81.0	81.0	83.0	82.0	82.0	81.0	78.0
After 5 minutes		74.0		81.0		75.0		75.0

TABLE 3

Subject R., weight, 58 kgm.

	FIRST WEEK	SECOND WEEK	THIRD WEEK		FOURTH WEEK	
	Ordinary diet	High protein	Low protein		High protein	
Average number of steps per day	14,980	13,145	16,415		14,965	
Urine N (average) grams	12.5	18.9	12.3		18.9	
Maximum-minimum	14.9–10.1	19.6–18.0	14.3–10.1		19.7–17.6	
	a. m.	*a. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk	20.9	22.0	21.8	19.1	21.9	19.3
Time in minutes to make 1000 steps	9.03	9.14	9.04	9.48	9.07	9.75
Pulse:						
Start of exercise	70.0	69.0	69.0	72.0	70.0	71.0
End of exercise	96.4	96.0	91.0	95.0	94.0	101.0
Time in minutes of return to normal	1.17	1.25	1.37	1.82	1.55	2.05

TABLE 4

Subject S., weight 40 kgm.

	FIRST WEEK		SECOND WEEK		THIRD WEEK		FOURTH WEEK	
	Ordinary diet		High protein		Low protein		High protein	
Average number of steps per day	13,902		14,552		15,695		14,808	
Urine N (average) grams	12.36		15.78		7.89		15.77	
Maximum-minimum	14.07–9.34		18.65–13.44		8.99–7.31		16.88–13.15	
	a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk	28.2	28.5	28.0	28.5	27.3	27.8	27.4	27.5
Time in minutes to make 1000 steps	9.24	9.34	9.17	9.34	8.99	9.18	9.04	9.13
Pulse:								
Before exercise	85.0	85.0	84.0	85.0	80.0	81.0	80.0	81.0
End of minute	111.0	113.0	114.0	110.0	120.0	112.0	121.0	119.0
After 3 minutes	97.0	97.0	94.0	98.0	85.0	88.0	79.0	88.0
After 5 minutes	90.0	95.0	87.0	90.0		80.0		80.0

SUMMARY

The results show that the presence or absence of meat from the dietary, during periods as long as one week, has no demonstrable effect upon the capacity of doing an amount of work so graded as to reach the limit of the physical capacity during a short period of time. This accords with the doctrine of Chittenden (2). There was a distinct and uniformly present sense of sleepiness for 2 or 3 hours during the afternoon period following the ingestion of 300 grams of meat. Removal of meat from the dietary for a period of one week did not diminish the sense of well-being in the individuals investigated. The well-nigh universal opinion that meat ingestion is important for the maintenance of physical strength is not to be disregarded, but the experimental evidence in favor of this conception has yet to be produced.

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THE GASTRIN THEORY PUT TO PHYSIOLOGICAL TEST[1]

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In 1906 Edkins (1) discovered that 0.4 per cent HCl extracts of pyloric mucous membrane when injected intravenously caused a secretion of gastric juice. This observation with certain qualifications has been confirmed by every investigator who has repeated the procedure and is, without question a fact. With the exception of the cardiac mucous membranes, Edkins believed this to be an action specific for the pyloric mucous membrane and he termed the active substance of the extract "gastric secretin." Gross (2) in the same year offered evidence that led him to believe that the mucosa of the pyloric portion of the stomach elaborated a "gastric hormone" when food substances were brought into contact with it. Popielski (3) in a series of papers (1909–1913) questioned the specificity of pyloric mucosa extract, stating that the stimulating action was due to the more fluid nature of the blood and to vasodilatation and could be obtained from tissue extracts in general. Ehrmann (4) reported that acid extracts of pyloric, fundic and duodenal mucosa caused a flow of gastric juice when injected subcutaneously. Einsmann (5) reported stimulation of gastric secretion to follow the injection of extracts of pyloric mucosa, duodenal mucosa, liver jejunum, ileum and pancreas. Tomaszewski (6) found a gastric secretogogue to be present in the pyloric and fundic mucosa. Keeton and Koch (7) found gastrin to be distributed throughout the gastric mucosa, in the duodenum, the esophagus and brain. Luckhardt, Keeton and Koch (8) found secretogogue action in extracts of the duodenum, liver, pancreas, thyroid and Armour's scale pepsin. Rogers, Fawcett et al (9) reported marked secretogogue action in extracts of thyroid, liver, parathyroids, spleen, pancreas, thymus and pineal gland. Luckhardt, Keeton and Koch (8), however, found the extracts of the spleen, thymus, muscle, gastric juice and fibrin to be inactive. Ivy and Oyama (16) found that gastric mucus did not contain a secretogogue. Luckhardt, Keeton and

[1] Preliminary report: Proc. Amer. Physiol. Soc., Chicago, 1920.

Koch (10) also found that histamine when injected subcutaneously will stimulate gastric secretion and which in many respects is very similar to the stimulation produced by gastrin.

So the bulk of the evidence at hand shows that the gastric secretogogue in the extract of pyloric mucosa is not specific for the pyloric and cardiac mucous membranes but has a wide distribution, being present "in relatively high concentrations," according to Koch, Luckhardt and Keeton (10) "in the same organs in which ammonia is generally most prominent immediately after death."

With the exception of the work of Gross (2) in 1906 and of Edkins and Tweedy (11) in 1909, all of the investigations of this problem have been of a pharmacological nature, *i.e.*, extracts have been prepared and injected parenterally and their effects on gastric secretion noted.

It is generally recognized that as a rule it is not correct to assume that pharmacological action denotes physiological significance. Because an extract of the pyloric mucous membrane when injected parenterally stimulates gastric secretion, it does not follow that the active principle of the extract is physiologically important or significant.

In accordance with this principle Edkins and Tweedy (11) devised a method which enabled them to functionally separate the pyloric from the fundic end of the stomach and reported that when "different substances (HCl, dextrin, dextrose, meat extracts, peptone solution) were placed in the pyloric region of the stomach or in the duodenum in all cases the fundus responded by marked secretion." These investigators divided the stomach into two chambers by means of a partition which consisted of a rubber balloon, shaped like a pulley wheel, which was inserted through the pyloric orifice from the duodenum. The balloon when in position in the stomach was inflated and tied in place by passing a ligature about the stomach outside the muscle wall but beneath the blood vessels, just opposite the groove in the pulley-shaped balloon, thus dividing the stomach into two water-tight compartments. Thirty cubic centimeters of normal saline solution at 37°C. were then introduced into the fundic chamber, after it had been washed out, and the different substances were then introduced into the pyloric chamber at a pressure of 10 cm. water pressure. This was allowed to remain for 1 to 2 hours when the 30 cc. of normal saline solution were removed from the fundic compartment and titrated for degree of acidity. They report acid values varying from 0.0058 per cent to 0.16 per cent. Cats were used. At no place in their paper do they state that controls were made, that is, experiments in which normal saline solution was placed in the fundic

compartment and nothing in the pyloric compartment and 1 to 2 hours later titrating the normal saline solution for acidity. In other words, they failed to take into consideration the possibility of a continuous secretion of gastric juice. The only reason that we are able to assign for omitting controls is a statement Edkins makes in a previous article (1) to the effect that he was "able to show that normal saline solution introduced into the stomach would remain for a prolonged period (1 to 2 hours) unabsorbed and without change in reaction." This statement was based on some work he did in 1892 on cats under chloroform anesthesia and a hypodermic injection of $\frac{1}{2}$ grain of morphine and $\frac{1}{13}$ grain of atropine. And even when the cats were under this atropinized condition his protocols report that two out of five cats experimented upon (cats XI and XII) showed an acid reaction of the normal saline solution that had remained in their stomachs from 1 to 2 hours. The two cats in which the acid reaction occurred had food in their stomachs previous to washing it out, which is a factor of importance, as will be pointed out in our experimental work. Further, Edkins states (1) that "atropine does not diminish the reaction of an animal to this excitant" (gastric secretin) which has been shown to be wrong by Keeton, Luckhardt and Koch (12) in Pavlov pouch dogs and by Maydell (17), and these latter results have been confirmed in the course of our work. Hence this phase of the work of Edkins can hardly be considered conclusive.

Sokolov (13), working in Pavlov's laboratory on animals with a Pavlov pouch, an "obstructed" stomach, a duodenal and gastric fistula with a rubber tube connecting the two fistulae reported that food substances when injected into the duodenum of such an animal would not exert any influence on the gastric glands, but when injected into the "obstructed" stomach and retained there, secretion of the Pavlov pouch would occur. These results were interpreted as demonstrating that the secretion was excited by reflex effect from the gastric mucous membrane and that the chemical secretion of gastric juice is caused by an effect proceeding mainly from the inner surface of the stomach and not from the intestine (18).

Gross (2) also working in Pavlov's laboratory on an animal preparation similar to that of Sokolov, with the exception that the obstruction was made at the junction of the fundic mucosa and pyloric mucosa, obtained results that partially contradicted the findings of Sokolov and drew conclusions from his results that supported the "gastrin theory" of Edkins. The results of Gross show when meat extract is injected into the fundic portion of the stomach and retained there, no stimulation

of secretion results in the Pavlov pouch, but when the meat extract is injected into the duodenum through the duodenal fistula that a stimulation of the gastric glands results. Gross explains this contradiction of Sokolov's findings by assuming that the meat extract he (Gross) injected into the duodenum flows back into the pyloric antrum and there produces its effect. This assumption we do not believe to be plausible because in our experience the pyloric antrum readily and rapidly expels its contents, as we shall point out later in our experimental work. A second assumption is also necessary and that is that the elaboration of "gastrin" actually does occur when meat extract is in contact with the pyloric mucosa, which has not yet been proved.

Fig. 1. *a*, abdominal wall; *b*, Pavlov pouch; *c*, gastro-duodenostomy; *d*, pyloric pouch.

Experimental: In the course of studies on the physiology of the pyloric secretion, animals with two gastric pouches (14), one a Pavlov pouch and the other a pouch of the entire pyloric antrum, were prepared (see fig. 1) so that the secretions from the fundic and pyloric mucous membranes could be collected simultaneously. It occurred to us that such an animal might be used to demonstrate the reality of the "gastrin theory," in which we were firm believers, because it was possible to apply substances to the pyloric mucosa in healthy or physiological animals and to observe changes in the secretion of the Pavlov pouch.

Method: Our method consisted in collecting two to three hours of continuous secretion (24 hours after the last meal) from the Pavlov pouch and then in applying the various substances for a period of 2

hours to the mucosa of the pyloric pouch and at the same time continuing the collection of secretion from the Pavlov pouch. Application of the substances was made by injecting the substances into the pouch and then plugging the orifice with cotton, this being repeated every 3 to 5 minutes. In order to insure continuous contact, as the pouch would contract and expel its contents, another device was made which alleviated this trouble.

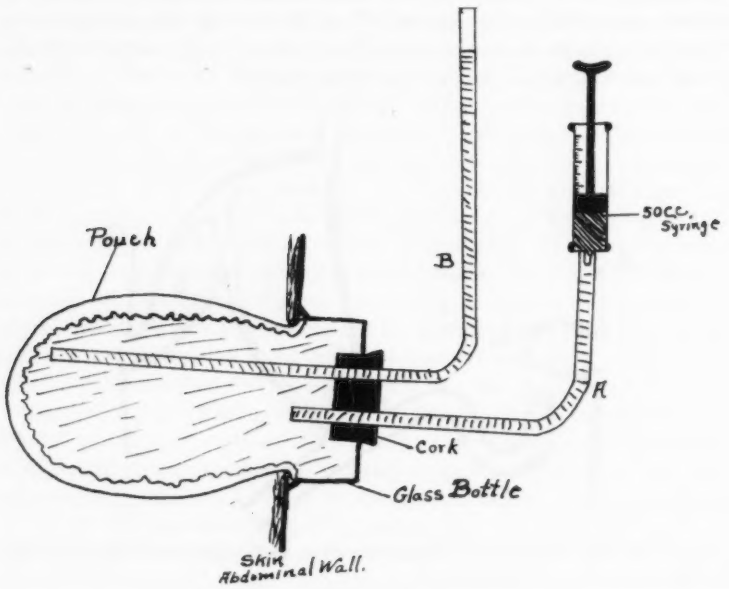

Fig. 2

The device consists of a glass bottle, a cork and a glass tube arranged as in figure 2. The solution to be applied is put into a 50 cc. syringe and injected slowly through tube *A*, so that all the air will be displaced from the bottle and the pouch through tube *B*, until the solution stands in tube *B* above the level of the pouch. The level of the solution in tube *B* and in the syringe varies with each respiration and is raised markedly with each contraction of the pouch. When the musculature of the pouch relaxes again the displaced fluid passes back into the pouch.

Four out of six dogs subjected to our operation lived in good health for 1 month or longer. The first dog operated only lived 16 days and

only a few tests were made on him. The second dog lived 3 weeks, the third dog 2 months, the fourth dog 5 weeks, the fifth dog 4 months and the sixth dog 7 months. Dog VI died 1 month after making a duodenal fistula. All of the dogs died of a similar trouble, the cause of which we have no definite explanation, the chief symptoms being loss of weight and anorexia, beginning 10 to 15 days prior to death—all dogs dying of intercurrent pneumonia. Anemia, associated with bleeding of the gums, was present in all of the animals prior to death. At autopsy no lesions were found other than broncho-pneumonia, which was assigned as the immediate cause of death.

Results: All of the dogs at the time the various substances were applied to the pyloric mucosa reacted normally to a standard meal of meat and to a deep injection of gastrin. In dog I only three tests were made. In all the other dogs many tests were performed using $\frac{N}{10}$ HCl, gastric juice, gastrin, fresh meat extract and Liebig's meat extract. Dextrose and peptone solutions were used twice in one animal with negative results.

The results were negative for all of the substances used, table 1 demonstrating the absence of response when fresh meat extract was applied to the mucosa of the pyloric pouch and the routine of procedure followed in all of our experiments. Further tables of results are omitted because of their negative nature. We desire to emphasize that after the application of the substance under test to the mucosa of the pyloric pouch the animal was given or injected with gastrin in order to demonstrate that the gastric secretory mechanism of the animal at the time of application was normal. We also desire to call attention to the necessity of following the continuous secretion of the Pavlov pouch before and after a certain procedure in order to determine whether or not the procedure caused stimulation.

Repetition of Edkins and Tweedy's experiments. Having failed to demonstrate the reality of the "gastrin theory" with our "two gastric pouch" preparation, we sought an explanation. We were convinced that Edkins and Tweedy (11) did not adequately control their experiments as has been pointed out in the first part of our paper. The work of these investigators was then repeated.

Method: We at first attempted to use the technique devised by Edkins and Tweedy, but in inserting the balloon more trauma was produced than we thought would be good for the results of the experiment. So we used a more simple technique—one that is frequently used—which consisted in tying a ligature about the cardiac orifice, the pyloric sphinc-

ter and a third one at the transition between fundic and pyloric mucous membrane. The ligatures were passed beneath the blood vessels. Our only variation from the technique of the above investigators then was to dispense with the use of the balloon. All solutions were warmed to 37°C. before using and the stomach was washed out before the introduction of the normal saline solution. Light ether anesthesia was used.

Results: The results (table 2) demonstrate that there is an increased acidity of the normal saline solution that is put in the fundic chamber within the same limits as occurs when 15 cc. of meat juice or Liebig's

TABLE 1

*Fresh meat extract solution applied to mucosa of pyloric pouch *dog III*

PROCEDURE	TIME	PAVLOV POUCH SECRETION	TOTAL ACIDITY	FREE ACIDITY	REMARKS
	o'clock	*cc.*	*per cent*	*per cent*	
Continuous secretion	10–11	1.0	0.2188	0.0912	
Continuous secretion	11–12	1.7	0.3098	0.2553	
Continuous secretion	12–1	2.0	0.3098	0.2553	
Fresh meat extract solution applied to pyloric pouch	1–2	2.2	0.2553	0.2188	Extract of 1 pound of lean beefsteak
Application continued	2–3	2.1	0.1459	0.1094	
Continuous secretion	3–4	2.0	0.1641	0.0730	
Test meal					
First hour	4–5	4.1	0.2918	0.1842	½ pound of lean beef and 200 cc. water
Second hour	5–6	10.0	0.3737	0.2918	
Third hour	6–7	5.0	0.4193	0.3463	

* The fresh meat extract was made by boiling 1 pound of ground lean beefsteak for 5 minutes in 200 cc. of water and then expressing the juice. The mixture was raised to the boiling point slowly in from 20 to 30 minutes.

meat extract are put in the pyloric chamber. The slight increase in acidity that occurs in both cases we believe to be due to the continuous secretion of the stomach. It is to be recalled that the highest acidity reported by Edkins and Tweedy is 0.16 per cent which we believe to be within the normal, even though our highest per cent of acidity is 0.13 per cent. The results on the dog are the same as those on the cat with the interesting difference that the secretory mechanism in the cat is more resistant to ether anesthesia and the experimental procedure than in the dog.

ABSORPTION FROM THE MUCOSA OF THE PYLORIC POUCH. The question of absorption from the mucosa of the pyloric pouch must be considered as it is possible that absorption may not occur at as fast a rate

TABLE 2

PROCEDURE	ANIMAL NUMBER	TOTAL ACIDITY OF N. S. S. TAKEN FROM THE STOMACH	AVERAGE TOTAL ACIDITY OF N. S. S. TAKEN FROM STOMACH
		per cent	*per cent*
Six dogs: Control, 50 cc. N.S.S. placed in fundic chamber	1	0.018	
	2	0.036	
	3	0.082	
	4	0.027	0.046
	5	0.054	
	6	0.036	
Six dogs: 50 cc. N.S.S. placed in fundic chamber and 15 cc. of meat extract in pyloric chamber	11*	0.073	
	12	0.054	
	13	0.018	0.042
	14	0.027	
	15	0.045	
	16	0.036	
Eight cats: Controls 30 cc. N.S.S. placed in fundic chamber	1*	0.091	
	2	0.018	
	3	0.063	
	4*	0.136	0.072
	5	0.054	
	6	0.082	
	7	0.136	
	8	0.045	
Six cats: 30 cc. N.S.S. placed in fundic chamber and 10–15 cc. of meat extract in pyloric chamber	11	0.027	
	12	0.054	
	13	0.136	0.070
	14*	0.127	
	15*	0.127	
	16*	0.091	

* The stomach was full of food. In all cases the stomach was washed out with from 300 to 500 cc. of N.S.S. at 37°C.

as it does in the pyloric mucosa when it is in its normal anatomical position. It is possible that the procedure of making the pouch might have in some way altered absorption from the mucosa of the pouch.

This question cannot be answered directly for any of the solutions used in our experiments, because with present methods it is practically impossible to prove without question whether or not absorption of the substances applied occurred, with the possible exception of dextrose and acid. We have attempted to answer the question indirectly by comparing the rates of absorption of strychnine sulphate, pilocarpine hydrochloride and potassium iodide from the mucosa of the pyloric pouch and from the mucosa of the pyloric antrum in situ.

Results: Solutions of strychnine sulphate (3 per cent), pilocarpine hydrochloride (100 per cent) and potassium iodide (100 per cent) were used. They were applied to the mucosa of the pouch in the usual manner. In the case of the strychnine, the time of occurrence of increased reflexes and of spasms was observed. In the case of pilocarpine hydrochloride, the time of salivation was noted. When potassium iodide was applied, the time of its appearance in the saliva and gastric juice was noted,[2] the starch test being used. The solutions were applied to the mucosa of the pyloric antrum in its normal position by anesthetizing the dog, ligating off the pyloric antrum with two ligatures, which were placed beneath the blood vessels, and by injecting the solution into the lumen of the pyloric antrum by means of a syringe, after which the abdomen was closed. Care must be exercised to have the needle of the syringe clean and not to spill any of the solution on the serosa of the stomach. In the case of strychnine (10 cc. of a 3 per cent solution) the ether was discontinued and the time of occurrence of increased reflexes and of spasm was observed. In the case of pilocarpine (1 cc. of a 100 per cent solution, Wharton's duct was cannulated and the time of the beginning of salivation noted. In the case of potassium iodide (5 cc. of a 100 per cent), Wharton's duct was cannulated and the chorda-tympani nerve stimulated every 10 minutes and the time of the appearance of potassium iodide in the saliva was noted.

When strychnine is applied to the pouch, the reflexes are definitely increased in from 15 to 20 minutes and spasms appeared in from 20 to 25 minutes. When styrchnine is injected into the pyloric antrum in situ, increased reflexes occur in from 20 to 35 minutes and spasms appear in from 35 to 45 minutes. Pilocarpine when applied to the mucosa of the pyloric pouch, causes salivation in from 3 to 5 minutes and vomiting and defecation in from 5 to 7 minutes; when injected into the pyloric antrum in situ, it causes salivation in from 10 to 15 minutes. Potassium iodide, when applied to the mucosa of the pyloric pouch, makes

[2] "Two gastric pouch" dogs used.

its appearance in the gastric juice in from 15 to 30 minutes and in the saliva, provided the dog's salivation is stimulated by pilocarpine injection, in from 1½ to 3 hours. When potassium iodide is injected into the pyloric antrum in situ, it appears in the saliva in from 1 to 2 hours and in the "fundic" secretion in from 15 to 30 minutes.

These data demonstrate that the rate of absorption of the mucosa of the pyloric pouch has not been altered by the operative procedure and that the absorption of potassium iodide and of strychnine from the pyloric mucosa is comparatively slow.

SUMMARY

We made the "two gastric pouch" preparation in the hope of demonstrating the "gastrin theory." This theory maintains that food substances in contact with the mucosa of the pyloric antrum cause the formation of a hormone, gastrin, which is absorbed into the blood stream and carried to the glands of the "fundic" mucosa and stimulates them. Our results do not support the gastrin theory. We failed to obtain an increase in the secretion of the Pavlov pouch when various substances, including food extracts, were applied to the mucosa of the pyloric pouch, which should have occurred according to the "gastrin theory."

Not being able to find fault with our preparation—the animals having been proven to be physiological in every respect—and experiments, we repeated the work of Edkins and Tweedy and have demonstrated that they did not adequately control their experiments. This deficiency of proper controls has also been pointed out by other investigators (3), (4), (5), (6), (7), (8), (9) and confirmed by us with reference especially to the claims of the "specificity of gastrin."

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